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(54) Title: **COMPOSITION FOR THE ELIMINATION OF AUTOREACTIVE B-CELLS**

(57) Abstract: The present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragments(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes and/or capable of activating the complement system.

WO 02/16414 A2

Composition for the elimination of autoreactive B-cells

The present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragment(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes. Preferably, said composition comprises at least one (poly)peptide construct consisting of at least two domains, wherein said domain comprising an autoreactive antigen or (a) fragment thereof is MOG or (a) fragment(s) thereof and wherein said domain comprising an effector molecule is an anti-CD3 receptor or an Fc-part of an immunoglobulin. Described is also the use of the afore-mentioned (poly)peptide construct for the preparation of a pharmaceutical composition for the treatment and/or prevention of an autoimmune disease. In addition, the present invention relates to method for treating, ameliorating and/or preventing of an autoimmune disease.

Several documents are cited throughout text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference.

Autoimmunity results from the failure of the immune system in tolerating self-reactive lymphocytes, resulting in an adaptive immune response against self antigens. When such immune responses are sustained, they cause lasting tissue damage and are classified as autoimmune diseases.

Autoimmune diseases are generally divided into three types: B-cell dominant, T-cell dominant or combinational types. Pathogenic phenotypes of B-cell dominant autoimmune diseases are caused by circulating autoantibodies produced by autoreactive B-cells, while those of the T-cell dominant type are caused by direct tissue damage of activated T-cells against cells presenting autoreactive peptide-MHC complexes on their surface. Yet, these distinctions are not perspicuous, since B-cells and T-cells cooperate with and depend on each other in each type of autoimmune disease. Autoimmune diseases are classified as combinatorial when both autoreactive B- and T-cells contribute directly to the pathogenesis observed ("Immunobiology", 4th ed. (1999), Chapter 13 pp 489-536, Janeway, C.A., Travers, P., Walport, M., Capra, J.D. eds and "Harrison's Principles in Internal Medicine", 14th ed, Fauci, Braunwald, Isselbacher, Wilson, Martin, Kasper, Hauser, Longo, eds).

The pathogenic effects of autoreactive B cells are caused by the secreted autoreactive antibodies. Antibody-mediated autoimmune diseases can be differentiated into two major groups based on their immunopathogenic mechanism. The first group comprises autoimmune responses against cell-surface or extracellular matrix antigens, while the second group consists of immune-complex diseases.

Examples of the first group of antibody-mediated autoimmune diseases are autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, myasthenia gravis, Goodpasture's syndrome, immunologically mediated blistering diseases like Pemphigus vulgaris and pemphigus foliaceus, and acute rheumatic fever. Examples of the second group comprise mixed essential cryoglobulinemia, subacute bacterial endocarditis, and several rheumatic autoimmune diseases.

Current treatment options for antibody-mediated autoimmune diseases include small molecule anti-inflammatory and immuno-suppressive agents, plasmapheresis, surgical treatments and/or cytokine administration.

For example, pemphigus vulgaris and pemphigus foliaceus are usually treated with glucocorticoids and, in some cases, also with immunosuppressive agents. Myasthenia gravis treatment options include anticholinesterase medications, immunosuppressive agents, thymectomy, plasmapheresis or intravenous unspecific

immunoglobulin. Multiple sclerosis treatments include interferon beta, glucocorticoids, plasmapheresis.

Yet, current therapies for autoimmune diseases/disorders are not selective, treat essentially only symptoms and/or lead to broad immuno-suppression.

Experimental approaches to therapy include removal of the entire B cell compartment using monoclonal antibodies against pan-B cell surface antigens (CD19, CD20), or even pan-leucocyte antigens (CD52). Anti-CD52 monoclonal antibodies have been tested in clinical trials for their ability to ameliorate conditions of patients with multiple sclerosis. However, the indiscriminate elimination of B and T cells using anti-CD52 monoclonal antibody leads to a substantial release of pro-inflammatory cytokines, contributing to a progressive phase of disability (Coles (1999) *Lancet* 354, 1691-1695; Coles (1999) *Ann. Neurol.* 46, 296-304). Thus, highly selective removal of only the autoreactive B cells is needed to prevent the disastrous side effects. Other pan-B cell antibody based approaches include immuno-toxins (WO 96/36360). Recently, an approach combining an autoantigen with an immunotoxin has been tested in vitro. The construct was a desmoglein3 (dsg3)-toxin fusion protein for the treatment of experimental pemphigus vulgaris (Proby, *Brit. J. Dermatol.* (2000) 142, 321-330). This approach, however, lacks efficacy. In Proby (2000), a cytotoxic effect of the dsg3-PE toxin was determined in vitro at concentrations of 50 microgram/ml. Assuming a distribution in blood and lymph fluid (about 10 l volume total) and an average weight of 70 kg, such a concentration would correspond to a dose of about 5000 to 7000 micrograms/kg. In contrast, the maximal tolerated dose (MTD) in man for an anti-CD25scFv-PE toxin (LMB-2) compound was determined at 63 micrograms/kg. Side effects included elevated transaminase levels and cardiomyopathy (Kreitman, (2000) *J. Clin. Oncol.* 18, 1622-1636). Similar MTDs were determined for scFv-toxin LMB-1, an anti-LeY scFv-PE toxin compound (75 microgram/kg; Pai, (1996) *Nat. Med.* 2, 350-353). Thus, the doses theoretically required for specific activity by dsg3-PE toxin are about 100 times higher than the MTD determined for PE toxin compounds. It is conceivable that further harmful toxicity could result from binding of the autoantigen-toxin construct to anti-dsg3 autoantibodies. The complex could then be bound and

internalized by any kind of FcR-bearing immune cells, killing thereby non-autoreactive immune cells and leading to a general immunosuppression.

In myasthenia gravis, approaches to downregulate autoimmune reactions using intact or portions of the acetylcholine receptor (AChR, Achr) has been the main goal of multiple studies in animal models. However, AChR is highly immunogenic and thus, frequent administrations of the molecule might lead to an immune response rather than tolerance induction. Therefore, short peptides that represent T cell epitopes of the AChR and especially altered T cell epitopes with less immunogenic potential than the native protein were tested in order to provide for an approach to therapy. In detail, dominant T cell epitope peptides of the Torpedo AChR were injected either before immunization with the Torpedo AChR or after priming suppressed disease manifestations. However, at least one of these studies reported lack of ability of the peptides to treat an ongoing disease in an animal model (Karachunski, (1999) *J. Neuroimmunol.* 93, 108-121)

Approaches to treat multiple sclerosis include treatments which affect the overall immune system, like treatment with anti-inflammatory agents, comprising azathioprine, cyclophosphamide, prednisone, corticosteroids, cyclosporin A, calcineurin, rapamycin, beta-interferon ("Harrison's Principles of Internal Medicine", 14th edition, McGraw-Hill publisher, 2415-2419; Wang, *J. Immunol.* 165 (2000), 548-557). In addition, a number of non-specific treatments are administered that may improve the quality of life including physical therapy and psycho-pharmacological agents. Experimental approaches include peptide ligands to block T cell epitopes (Holz, *J. Immunol.* 164 (2000), 1103-1109; Krogsgaard, *J. Exp. Med.* 191 (2000), 1395-1412) and TNF alpha inhibitors (Klinkert, *J. Neuroimmunol.* 72 (1997), 163-168).

Autoreactive B cells occur at a very low frequency. For example, in a frequency of about 10^{-6} to 10^{-7} of total B cells circulating in the blood of individuals suffering from multiple sclerosis. This low frequency has been a major impediment in the isolation of such cells from patients. Elimination of autoreactive B cells in vivo can therefore only be monitored indirectly via the determination of autoreactive antibody titers and

is further complicated by the long half-life of antibodies in serum. Direct in vitro testing of the proposed concept for the elimination of autoreactive B cells using human ex-vivo samples from patients suffering from B cell mediated autoimmune disease has not been possible due to the low abundance of these cells.

Therefore, the technical problem underlying the present invention was to develop and to provide for means and methods for preventing, treating and/or ameliorating antibody-mediated autoimmune diseases/disorders.

The solution to said technical problem is achieved by the embodiments characterized in the claims.

Accordingly, the present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragment(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes and/or capable of activating the complement system.

The present invention is based on the surprising finding that compositions as described herein are capable of selectively eliminating autoreactive B cells and thereby removing the pathological cell causing an autoimmune disorder/diseases.

Autoreactive B-cells are described herein above and are known to the skilled person as, e.g., illustrated in Immunobiology, Janeway and Travers, 1996 by Current Biology Ltd/Garland Publishing Inc.. B lymphocytes bear on their surface highly diverse receptors each of which is specific for a particular antigen and which together are capable of recognizing a wide diversity of antigens. The antigen receptor of B lymphocytes is a membrane-bound form of the antibody that these cells will secrete when activated, see, inter alia, p. 1:6 in Immunobiology, Janeway and Travers, 1996 loc. cit.. In the case of autoreactive B cells, these antigens bound to the antigen receptors are self antigens. Autoimmune diseases are mediated by

immune responses specific for self antigens; see p. 1:18 in Immunobiology, Janeway and Travers, 1996 loc. cit.. In particular and preferably, autoreactive B-cells are B-cells of all differentiation states, resting or activated, which carry a B-cell Ig-receptor on this cell surface and which – due to this feature – are capable of binding to/interacting with (a) specific autoantigen(s). B-cell specific markers are also known in the art and comprise the whole family of membrane bound Ig molecules, preferably IgM+, IgD+ or IgG+, preferably in combination with any of the following markers B220+ (CD45R+), CD19+, CD20+, CD22+, CD21+, CD38+, CD49c+, CD72, CD79 α , β +, CDw78+, MHC class II and CD43- (for resting B cells).

Here, it was surprisingly found that constructs comprising an autoreactive antigen or (a) fragment(s) thereof as described herein may be employed for the selective elimination of autoreactive B-cells and/or reduction of autoreactive immunoglobulins. This is surprising since the person skilled in the art would expect that the administration of autoantigen(s) and (a) fragment(s) thereof would lead to a more profound prevalence and/or disease state of autoimmune disorders.

The term "composition", in context of this invention, comprises at least one (poly) peptide construct as defined herein and/or at least one (poly) nucleotide comprising a nucleic acid molecule encoding for such one (poly) peptide construct. Said composition, optionally, further comprises other molecules, either alone or in combination, like e.g. molecules which are capable of modulating and/or interfering with the immune system. The composition may be in solid, liquid or gaseous form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). In a preferred embodiment, said composition comprises at least two, preferably three, more preferably four, most preferably (poly)peptide constructs (and/or nucleic acid molecules encoding said constructs) as described in the invention.

The term "selective elimination" as used in accordance with the present invention means elimination of the above mentioned autoreactive B-cells in vivo as well as in vitro. Said term also comprises ex vivo elimination, inter alia, by dialysis approaches. It is preferred that said selective elimination does not hinder the immunological

response and/or only minimally influences the natural immunological defense. Preferably, said elimination does not compromise the T-cell mediated immune response and/or does not interfere with non-autoreactive B-cells. It is preferred that said elimination is caused by cytolysis, most preferably said cytolysis is mediated by cytotoxic cells, like, macrophages, monocytes, granulocytes, (cytotoxic) T-lymphocytes, natural killer (NK) cells and/or lymphokine-activated killer (LAK) cells.

The term "at least one (poly)peptide construct" as employed herein above relates to at least one (poly)peptide construct, at least two, at least three, at least four or at least five (poly)peptide constructs which may be comprised in the composition of the present invention.

The term "at least two domains" as used herein above comprises at least two domains, at least three domains, at least four domains and at least five domains in accordance with the invention.

As employed in accordance with this invention, the term "autoreactive antigen or (a) fragment(s) thereof" means antigens or (a) fragment(s) thereof which are capable of elucidating and/or mediating an autoimmune response. Said fragment(s) thereof is/are preferably an epitope of said antigen. Preferably, said antigens and/or its fragment(s) comprise proteinaceous structures, yet, said autoreactive antigen or (a) fragment(s) may also comprise, either alone or in addition to said proteinaceous structures, inter alia, carbohydrate moieties or lipids. The term autoreactive antigen or (a) fragment(s) thereof" is not limited to antigens occurring in and/or deriving from the subjects own body (autologous and/or endogenous antigens) but furthermore comprises foreign molecules which are capable of eliciting an autoimmune-response by binding and/or interacting with molecules peculiar to one's own body (for example via hapten-carrier complexes). In addition, said term also comprises antigens, like microbial antigens/epitopes, that share properties, e.g. amino acid sequences, with mammalian molecules, e.g. proteins, and are capable of provoking an autoimmune-response. Examples of such antigenic mimicry are known in the art (see, inter alia, Paul, "Fundamental Immunology", Raven Press, 1989) and comprise exogenous antigens like, Streptococcal M protein, Klebsiella nitrogenase, Measels virus P3,

retroviral p30 protein. It is preferred that the composition of the present invention comprises a (poly)peptide construct comprising a domain with at least one autoreactive antigen or at least one fragment thereof. However, it is also envisaged that said (poly)peptide construct comprises a domain comprising more than one autoreactive antigens and/or fragments and/or epitopes thereof. Said domain comprising said autoreactive antigen or (a) fragment thereof may therefore comprise several autoantigens and/or fragment(s) thereof. In a preferred embodiment said domain comprises at least one, more preferred at least two, more preferred at least three, more preferred at least four and most preferred at least five autoreactive antigen(s) or (a) fragment(s).

The term "effector molecule capable of interacting with and/or activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes" relates, in accordance with this invention, to molecules capable of engaging, inter alia, lymphocytes and/or FcγR positive cells in effector mechanisms, like the cell-lysis and/or phagocytosis. Said lymphocytes and/or FcγR positive cells comprise the above mentioned NK-cells, macrophages, monocytes and/or granulocytes, as well as lymphokine-activated killer cells, neutrophils, eosinophils.

In accordance with the present invention, the term "effector molecule capable of activating the complement system" relates to effector molecules which are capable of activating the classical as well as the alternative complement pathway. Furthermore, said term relates to effector molecules capable of activating any other form of complement mediated lysis.

Useful "effector molecules" in accordance with the present invention are, inter alia, disclosed herein and exemplified in the appended examples.

In a preferred embodiment, the present invention relates to a composition wherein the above described (poly)peptide construct is a fusion (poly)peptide or a mosaic (poly)peptide. Said fusion (poly)peptide may comprise merely the domains of the (poly)peptide construct as described herein above as well as several (a) functional fragment(s) thereof. However, it is also envisaged that said fusion poly)peptide

comprises further domains and/or functional stretches. Therefore, said fusion poly)peptide can comprise at least one further domain, said domain being linked by covalent or non-covalent bonds. The linkage (as well as the construction of the (poly)peptide constructs comprised in the composition of the present invention), can be based on genetic fusion according to the methods known in the art (Sambrook et al., loc. cit., Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989)) or can be performed by, e.g., chemical cross-linking as described in, e.g., WO 94/04686. The additional domain present in the fusion poly)peptide may preferably be linked by a flexible linker, advantageously a (poly)peptide linker, wherein said (poly)peptide linker preferably comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the peptide, (poly)peptide or antibody or vice versa. Said linker may, inter alia, be a Glycine, a Serine and/or a Glycine/Serine linker. Additional linkers comprise oligomerization domains. Oligomerization domains facilitate the combination of two or several autoantigens or fragments thereof in one functional molecule. Non-limiting examples of oligomerization domains comprise leucine zippers (like jun-fos, GCN4, E/EBP; Kostelny, J. Immunol. 148 (1992), 1547-1553; Zeng, Proc. Natl. Acad. Sci. USA 94 (1997), 3673-3678, Williams, Genes Dev. 5 (1991), 1553-1563; Suter, "Phage Display of Peptides and Proteins", Chapter 11, (1996), Academic Press), antibody-derived oligomerization domains, like constant domains CH1 and CL (Mueller, FEBS Letters 422 (1998), 259-264) and/or tetramerization domains like GCN4-LI (Zerangue, Proc. Natl. Acad. Sci. USA 97 (2000), 3591-3595).

Furthermore, the (poly) peptide construct as described herein may comprise further domains, inter alia, domains which provide for purification means, like, e.g. histidine stretches.

It is also envisaged that the (poly) peptide construct as described herein comprises (a) further domain(s) which may function as immunomodulators. Said immunomodulators comprise, but are not limited to cytokines, lymphokines, T cell co-stimulatory ligands, etc.

Adequate activation resulting in priming of naïve T-cells is critical to primary immunoresponses and depends on two signals derived from professional APCs (antigen-presenting cells) like dendritic cells. The first signal is antigen-specific and normally mediated by stimulation of the clonotypic T-cell antigen receptor that is induced by processed antigen presented in the context of MHC class-I or MHC class-II molecules. However, this primary stimulus is insufficient to induce priming responses of naïve T-cells, and the second signal is required which is provided by an interaction of specific T-cell surface molecules binding to co-stimulatory ligand molecules on antigen presenting cells, further supporting the proliferation of primed T-cells. The term "T-cell co-stimulatory ligand" therefore denotes in the light of the present invention molecules, which are able to support priming of naïve T-cells in combination with the primary stimulus and include, but are not limited to, members of the B7 family of proteins, including B7-1 (CD80) and B7-2 (CD86).

In the light of the present invention, proteinaceous compounds providing the primary activation signal for T-cells can comprise, but are not limited to, anti-CD3-svFv fragments, anti-T-cell receptor svFv fragments or superantigens. Superantigens directly bind to certain subfamilies of T-cell receptor variable regions in an MHC-independent manner thus mediating the primary T-cell activation signal.

Furthermore, the invention also relates to the effector molecule as defined herein, wherein the T-cell co-stimulatory ligand is a cell surface molecule or a fragment thereof expressed on antigen-presenting cells (APC).

Additionally, the effector molecule as defined herein, binding to an APC, may be a T-cell co-stimulatory factor like B7-1 (CD80) or B7-2 (CD86), or adhesion proteins like LFA-3 (CD58), ICAM-1 (CD54), ICAM-2 or ICAM-3 or like the CD137-ligand.

The effector molecule defined herein above may have receptor or ligand function, and may be an immuno-modulating effector molecule or a fragment thereof. An immuno-modulating effector molecule positively and/or negatively influences the humoral and/or cellular immune system, particularly its cellular and/or non-cellular components, its functions, and/or its interactions with other physiological systems.

Said immuno-modulating effector molecule may be selected from the group consisting of cytokines, chemokines, macrophage migration inhibitory factor (MIF; as described, inter alia, in Bernhagen (1998), *Mol Med* 76(3-4); 151-61 or Metz (1997), *Adv Immunol* 66,197-223), T-cell receptors and soluble MHC molecules. Such immuno-modulating effector molecules are well known in the art and are described, inter alia, in Paul, "Fundamental immunology", Raven Press, New York (1989). In particular, known cytokines and chemokines are described in Meager, "The Molecular Biology of Cytokines" (1998), John Wiley & Sons, Ltd., Chichester, West Sussex, England; (Bacon (1998). *Cytokine Growth Factor Rev* 9(2):167-73; Oppenheim (1997). *Clin Cancer Res* 12, 2682-6; Taub, (1994) *Ther. Immunol.* 1(4),229-46 or Michiel, (1992). *Semin Cancer Biol* 3(1),3-15).

Particularly preferred are cytokines which are selected from the group consisting of interleukin(s), interferon(s), TNF(s) and VEGF (Veikkola *Semin Cancer Biol* 9(3), 211-20), wherein said interleukin(s) comprise, but are not limited to IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17 and IL-18, wherein interferon(s) comprise IFN- γ as well as IFN- β and IFN- α and wherein TNF(s) comprise members of the lymphotoxin superfamily like TNF- α and TNF- β (Gruss (1996) *Int J Clin Lab Res* 26(3),143-59). Other suitable cytokines are well known in the art and comprise, inter alia, GM-CSF, G-CSF, M-CSF. In a particular preferred embodiment, said immuno-modulating effector molecule is a chemokine and is selected from the group consisting of IL-8, Eotaxin, GRO α , GRO β , GRO γ , IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MIG, MIP-1 α , MIP-1 β , NAP-2, RANTES, I309, Lymphotactin, SDF-1 and C5a.

Further effector molecules may be selected from the group of neuroprotective proteins, such as the neurotrophic growth factors. Examples of such molecules are the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and/or the growth factors IGF-1 and bFGF, and/or the respective receptors of the aforementioned molecules (Connor B., *Brain Res. Brain Res. Rev.* 27 (1998), 1-39; Lewin, *Annual Review of Neuroscience* 19 (1996), 289-317; Tessarollo, *Cytokine Growth Factor Rev.* 9 (1998), 125-137; Snider, *Cell* 77 (1994), 627-638; Garcia-Estrada, *Brain Res.* 592 (1992), 3443-347; Lindsay,

Trends in Neurosciences 17 (1994), 182-190; Minichiello, Genes Development 10 (1996), 2849-2858). The therapeutic potential of NGF treatment in autoimmune encephalomyelitis has been shown by Villoslada et al. in the marmoset EAE model (J. Exp. Med. 191 (2000), 1799-1806).

Within the scope of the present invention are furthermore effector molecule comprising, inter alia, different immunomodulating effector molecules. Particularly preferred are effector molecules are cytokines, like IL-2 and GM-CSF.

The (poly)peptide constructs comprised in the composition of the present invention may be constructs which comprise domains originating from one species, preferably from mammals, more preferably from human. However, chimeric and/or humanized constructs are also envisaged and within the scope of the present invention.

In a particular preferred embodiment, the composition of the invention comprises a construct which is a cross-linked (poly)peptide construct. As mentioned herein above, said cross-linking may be based on methods known in the art which comprise recombinant as well as biochemical methods.

The present invention relates in a further embodiment to a composition as described herein above, wherein said autoreactive antigen or (a) fragment(s) thereof is selected from the group consisting of intracellular matrix proteins, extracellular matrix proteins, complement factors, nuclear antigens, cell surface receptors, nuclear receptors, lipids, soluble factors, membrane proteins, heat shock proteins, proteins with sequence similarity to microbial antigens, dietary components and proteins of intercellular structures.

In a more preferred embodiment, said intracellular matrix protein is selected from the group consisting of keratin, filaggrin, antiperinuclear factor 7.

So far, rheumatoid factor (RF), an IgM autoantibody directed against the Fc region of IgG2a, is still the only well-established serological disease marker for rheumatoid arthritis (Tighe (1997). in "Textbook of Rheumatology" W.B. Saunders Company, Philadelphia, PA. 241-249.). However, rheumatoid factor is not specific for

rheumatoid arthritis (RA) and is often negative in the early stages of the disease when a definite diagnosis is not always possible. In the past few years several new autoantibodies have been described which may be more specific for RA than rheumatoid factor. Among these are anti-A2/ RA33 antibodies (Hassfeld (1989) *Arthritis Rheum.* 32: 1515-1520; Hassfeld (1993) *Br. J. Rheumatol.* 32: 199-203), antikeratin antibodies and the antiperinuclear factor 7 (Youinou (1995) *Int. Arch. Allergy Immunol.* 107: 508-518; Sebbag (1995) *J. Clin. Invest.* 95: 2672-2679), and anti-Sa antibodies (Dèsprès, N., G. Boire, F.J. Lopez-Longo, and H.A. Menard (1994) *J. Rheumatol.* 21: 1027-1033). Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex (Skriner, *J. Clin. Invest.* 100 (1997), 127-135). Antibodies to the Sa antigen (streptococcal antigen) cross-react in an example of molecular mimicry with a poorly soluble human protein that is present in normal tissues and that is distinct from all previously described RA-associated autoimmune systems (Depres, *J. Rheumatol.* 21 (1994), 1027-1033).

Furthermore, the cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis (Simon (1993) *J. Clin. Invest.* 92,1387-93)

In yet another embodiment, the present invention relates to a composition as described herein above, wherein said extracellular matrix protein is collagen. It is particularly preferred that said collagen is collagen type IV or collagen XVII. In this context, the autoreactive antigen or (a) fragment thereof may also be the non-collagenous domain of collagen.

Goodpasture's syndrome results from antibodies directed against collagen type IV, in particular the non-collagenous domain of the basement membrane collagen type IV (Butkowski et al. (1987) *J. Biol. Chem.* 262, 7874-7877; Saus et al. (1988) *J. Biol. Chem.* 263, 13374-13380). The clinical manifestations of Goodpasture's syndrome are glomerulonephritis and pulmonary hemorrhage (Wilson, C., and Dixon, F. (1986) in the kidney (Berner, B., and Rector, F. eds) 3rd Ed., pp800-889, W.B. Saunders Co., Philadelphia).

Serum levels of autoantibodies to hemidesmosomal collagen XVII / BP180 were reported to correlate with disease activity in patients with bullous pemphigoid (see herein below).

In a further embodiment of the present invention, the above mentioned complement factor is C5. It has been shown that the complement factor C5 is a potent autoantigen in rheumatoid arthritis (Volkman, J. Immunol. 158 (1997), 693-706; Grant, Cell Immunol. 167 (1996), 230-240)

Furthermore, in a more preferred embodiment, the present invention relates to the above described composition wherein said nuclear antigen is selected from the group consisting of DNA, histones, snRNPs, topoisomerase I, ro (SS-A-Ro), la (SS-B-La), Scl-70, centromer protein (CENP), large ribonuclear particles (RNPs), AL Sm proteins, tRNA synthetase and Ku antigen.

A characteristic feature of rheumatic autoimmune diseases such as Systemic lupus erythematosus (SLE), progressive systemic sclerosis, polymyositis, mixed connective tissue disease (MCTD), or RA is the occurrence of autoantibodies to intracellular antigens (von Muehlen (1995) Semin. Erthritis Rhem. 24, 323-358; Peng (1997) in "Textbook of Rheumatology." W.B. Saunders Company, Philadelphia, PA. 250-266). For reasons which are not yet fully understood, these autoantibodies are often directed to components of large ribonucleoprotein (RNP) structures such as the ribosome or the spliceosome (van Venrooij (1995) Curr. Opin. Immunol. 7: 819-824). Some of these autoantibodies specifically occur in only one disease, which makes them very useful for diagnosis and treatment. Thus, autoantibodies to double-stranded DNA or to the Sm antigen (Brahms, JBC 275 (2000), 17122) are highly specific for SLE, autoantibodies to topoisomerase (anti-Scl70) (Mukai, J. Rheumatol. 20 (1993), 1594-1497, van Venrooij, Curr. Opin. Immunol. 7 (1995), 819-824) are exclusively detected in patients with progressive systemic sclerosis, and autoantibodies to tRNA synthetases (e.g., anti-Jo1) (Marguerie, Q.J. Med. 77 (1990), 1019-1038) occur only in patients with poly- or dermatomyositis.

Systemic lupus erythematosus (SLE) is an idiopathic autoimmune disease in which self-reactive autoantibodies (Cabral (1997); Curr. Opin. Rheumatol. 9, 387-392) cause disease either by directly binding to self-antigens or following the deposition of

antibody-antigen immune complexes in blood vessels leading to vasculitis, glomerulonephritis and arthritic tissue damage (Rothfield (1985) in "Arthritis and Allied Conditions", Lea & Febiger, Philadelphia, pp. 911-935). The estimated prevalence of SLE in the U.S. is 45/100,000, with the peak incidence in women ages 20-40 (Hochberg (1997) in "Dubois' Lupus Erythematosus", Williams & Wilkins, Baltimore, pp. 49-65). Proteins cleaved by interleukin-1 beta converting enzyme family proteases during apoptosis, as well as the Sm proteins B/B', D1, and D3 of the spliceosome, are common targets for autoantibody production in patients with systemic lupus erythematosus (SLE) (Brahms (2000) JBC 275, 17122 ff.). In addition, immune responses to SS-A 52-kDa and 60-kDa proteins and to SS-B 50-kDa protein have been shown in mothers of infants with neonatal lupus erythematosus (Yukiko (2000) Br. J. Dermatol. 142, 908-912). Photosensitivity of lupus erythematosus was correlated with the expression of SS-A/Ro and SS-B/La antigens in skin biopsy specimens of patients (Ioannides (2000) Arch Dermatol 136, 340-346). Wang et al. (J. Clin. Invest. (1999) 104, 1265-1275) have identified a novel 75-kDa phosphoprotein associated with SS-A/Ro and distinct human autoantibodies directed against it. Circulating anticentromere CENP-A and CENP-B antibodies were identified in patients with diffuse and limited systemic sclerosis, systemic lupus erythematosus, and rheumatoid arthritis (Russo (2000) J Rheumatol. 27, 142-148). Ku is a heterodimeric protein composed of approximately 70- and approximately 80-kDa subunits (Ku70 and Ku80) originally identified as an autoantigen recognized by the sera of patients with autoimmune diseases (Tuteja (2000) Crit Rev Biochem Mol Biol 35, 1-33).

The present invention relates furthermore to a composition as described herein wherein said autoreactive antigen or (a) fragment thereof is a cell surface receptor and wherein said a cell surface receptor is selected from the group consisting of TSH-receptor, Ach-receptor, asialo-glycoprotein receptor and platelet integrin GpIIb:IIIa.

Grave's disease is an autoimmune condition characterized typically by hyperthyroidism, thyroid hyperplasia, and additional signs of ophthalmopathy, pretibial myxedema, or acropachy. The pathophysiological mechanisms responsible

for thyrotoxicosis and thyroid hyperplasia are attributed to autoantibodies directed against the thyrotropin receptor (TSHr). Said antibodies activate the TSH receptor, which results in cAMP-dependent stimulation of thyrocyte function and growth (McKenzie (1995) in "Endocrinology". ;. W.B. Saunders Co. Philadelphia, PA; pp 676-711)

Autoantibodies directed against the acetylcholine receptor (Ach-receptor) are involved in Myasthenia gravis, an autoimmune disease which leads to a reduction of the number of (Ach-R) at the muscular motor endplate (see, inter alia, Heitmler (1999) Semin. Thorac Cardiovasc. Surg., 11, 41-46 or Atassi (1997) Crit. Rev. Immunol.;17, 481-495). In addition, autoantibodies directed against the human asialo glycoprotein receptor are described in autoimmune hepatitis (Bojic (1997) Med. Pregl. 50, 363-8)

Furthermore, autoantibodies against platelet integrin GpIIb:IIIa are the cause for autoimmune thrombocytopenic purpura resulting in abnormal bleeding (Beardsley and Ertem (1998) Transfus. Sci. 19, 237-244). Myasthenia gravis is caused by autoantibodies against the nicotinic acetylcholine receptors (AchR) leading to the downregulation of receptors and complement dependent lysis of the neuromuscular junction. The consequences are defects in neuromuscular transmission, culminating in weakness and fatigue of skeletal muscles in MG patients (Fambrough (1973) Science 182, 293-295; Kao (1977) Science, 196, 527-529; Heinemann (1977) Proc. Natl Acad. Sci. USA 7, 3090-3094).

In an even more preferred embodiment, the present invention provides for a composition wherein the soluble factor mentioned herein above is selected from the group consisting of I-antigen, Rh-blood group factor, 21-hydrolase enzyme, glutamic acid decarboxylase (GAD), insulin, (ICA) 512, ICAP-69, (tissue) transglutaminase (tTG), transaldolase, S100beta, oxidized low-density lipoprotein (ox-LDL), crystallin, CNPase, proteinase 3 and type I antigen.

Autoimmune hemolytic anemia is caused by antibodies against Rh blood group antigens and type I antigens, destroying red blood cells and resulting in anemia (Leddy (1993); J. Clin Invest. 91, 1672-1680; Leddy (1994); 84, 650-656)

Celiac disease, also referred to as gluten sensitive enteropathy, is characterized by IgA autoantibodies against anti-tissue transglutaminase and antiendomysial antibodies (EMA). (Lock, R.J. (1999) J Clin Pathol 1999 Apr;52(4):274-277; Rose, C. (1999) J Am Acad Dermatol 41, 957-961; Vitoria, J.C. (1999) J Pediatr Gastroenterol Nutr 29, 571-574; Schuppan, D. (2000) Gastroenterology 119, 234-242).

Auto-antibodies to oxidized low-density lipoprotein (ox-LDL) are thought to play a pivotal role in the pathogenesis of atherosclerosis and can serve as a marker of coronary artery disease in patients with familial hypercholesterolaemia (Paiker (2000) Ann Clin Biochem 37, 174-178)

Insulin-dependent diabetes mellitus (IDDM) is traditionally classified as a T-cell dependent autoimmune disease. However autoreactive antibodies are present in patients with IDDM. Contributions of the humoral response to the onset of disease and disease progression of IDDM was shown (Bonifacio (2000) Diabetes 49, 202-208; Coleman (2000) Diabetologia 43, 203-209; Rulli, M. (1999) Autoimmunity 31, 187-193). Also a connection between autoantibodies to diabetes mellitus and celiac disease was shown (Galli-Tsinopoulou A (1999) Horm Res 52, 119-124). A major target of autoimmunity in preclinical type 1 diabetes is glutamic acid decarboxylase, GAD, specific examples are GAD65, GAD67 (Bonifacio (2000) Diabetes 49, 202-208). Islet cell autoantigen (ICA) 512 is an autoantigen of insulin-dependent diabetes mellitus (IDDM) which is homologous to receptor-type protein tyrosine phosphatases (++PTPases). ICA 512 is an intrinsic membrane protein of secretory granules expressed in insulin-producing pancreatic beta-cells as well as in virtually all other peptide-secreting endocrine cells and neurons containing neurosecretory granules (Solimena (1996) EMBO J 15, 2102-2014). Other autoantigens in IDDM are ICAp69, (Karges (1996) Diabetes 45, 513-521). Anti-insulin antibodies were shown to be linked to the onset of diabetes (Yu (2000) Proc Natl Acad Sci U S A 97, 1701-1706)

Granulomatosis also known as Morbus Wegener (Hewis, Curr. Opin. Rheumatol. 12 (2000), 3-10) is caused by autoantibodies against proteinase 3, a constituent of neutrophil azurophilic granules.

In addition, the present invention provides for a composition as described herein above, wherein said heat shock protein is selected from the group consisting of alpha B-crystallin, Hsp27, HSP70 and HSP60. Alpha B-crystallin and Hsp27 have been implicated in multiple sclerosis (Agius, *Acta Neurol. Scand.* 100 (1999), 139-147). Furthermore, antibodies to mycobacterial heat shock proteins bind to human myelin and to oligodendrocytes recognizing human autoantigens, including HSP70 and myelin protein CNP (Salvetti, *J. Neuroimmunol.* 64 (1996), 143-153; Salvetti, *J. Autoimmun.* 5 (1992), 691-702; Birnbaum, *Ann. N.Y. Acad. Sci.* 835 (1997), 157-167; Jones, *Immunol. Today* 14 (1993), 115-118). Antibodies against *Escherichia coli* and chlamydial Hsp60 were shown to crossreact with human HSP60 (Mayr, *Circulation* 99 (1999), 1560-1566). Retinal autoantigens include Hsc70 (Ohguro, *Invest. Ophthalmol. Vis. Sci.* 40 (1999), 82-89).

As will be discussed in detail herein below, several proteins have been identified that can act as autoantigens in multiple sclerosis, including the soluble proteins CNPase, Transaldolase, S100 β , B-crystallin and other heat shock proteins (see, Schmidt (1999) *Multiple Sclerosis* 5, 147-160 or Bajramovic (2000) *J Neuroimmunol* 106, 14-22)

The present invention relates, in a more preferred embodiment, to a composition as described herein above, wherein said membrane protein is selected from the group consisting of PLP, MAG, MBP, MOG, Golgi-proteins, cytochrome P450 (CYPs) and UDP-glucuronosyltransferase (UGTs).

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) of autoimmune origin, characterized by focal demyelination, loss of oligodendrocytes, and astrocytic scar formation in advanced stages of the disease. Histopathologically, acute inflammatory lesions are characterized by infiltrating lymphocytes and macrophages scattered throughout the periventricular white matter, spinal cord, brainstem and optic nerves. In the later stages of the disease, vascular infiltrates are less prominent, and loss of myelin and oligodendrocytes predominates. While MS has been widely classified to be a majorly T-cell mediated disease, it is

now recognized that MS has both a T-cell and a B-cell component (Ewing (1998) *Immunology and Cell Biology* 76, 47-54; Weckerle (1999) *Nature Medicine* 5, 153-154; Genain (1999) *Nature Medicine*, 5, 170-175; Schmidt (1999) *loc.cit.*; Lindert (1999) *Brain* 122, 2089-2099). Eventually, myelin breakdown as the hallmark of the disease is brought about by the combined effects of autoantibodies against myelin proteins, complement activation, cytotoxic cells and cytokine-induced toxicity.

As mentioned herein above, several soluble proteins have been identified as being involved as autoantigens in multiple sclerosis, including the above discussed soluble proteins like, CNPase, transaldolase, S100 β or B-crystallin and/or other heat shock proteins. However further proteins play major roles in MS. These proteins comprise myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), PLP, MAG, (Schmidt (1999) *loc.cit.*). While it was long thought that the major myelin component MBP was the major target antigen in MS, studies of the experimental animal model of MS, experimental autoimmune encephalomyelitis (EAE), have suggested that other candidate autoantigens may be more relevant to the disease. Indeed, the MOG protein, a minor constituent of myelin sheaths (0.05% of total myelin) and exclusively expressed as a cell-surface protein on their outermost surface layer, has been shown to be the only single protein able to induce Chronic relapsing EAE (CREAE) in the Lewis Rat. CREAE is thought to be the most appropriate animal model for MS. MOG is a membrane glycoprotein found predominantly in the outermost lamella of the myelin sheaths that wrap and insulate axons in the CNS. It is a minor protein component of myelin representing just 0.05% of the total protein content, in contrast to other myelin proteins with encephalitogenic potential such as proteolipid protein (PLP) making up 20% of myelin protein. Nonetheless, immunization with MOG induces severe chronic EAE characteristically accompanied by strong inflammatory and de-myelinating lesions of the CNS (Iglesias, Glia, in press (2001). It was demonstrated that MOG-induced EAE comprises an important pathogenic antibody component (Schluesener, *J. Immunol.* 139, (1987), 4016-4021 and Litztenburger, *J. Exp. Med.* 188, (1998), 169-180). Hence strategies aimed at treating MS should not neglect the targeting and possible elimination of MOG-specific B cell clones. While transfer of MOG-specific T-cells has no apparent consequences in the recipient animal, co-transfer of T-cells and antibodies against MOG can induce EAE in animal models, resembling multiple sclerosis in humans

(Stefferi (1999) J. Immunol. 163, 40-49). These findings suggest that auto-antibodies against MOG play a central role in disease development and progression. Gold-labeled MOG peptides have been shown to bind specifically to disintegrating myelin around axons in lesions of acute multiple sclerosis and in MOG-induced EAE in the marmoset model for MS, providing direct evidence that autoantibodies against MOG mediate target membrane damage in central nervous system demyelinating disease (Genain (1999) Nature Medicine, 5, 170-175; Raine (1999), Ann. Neurol. 46, 144-160). In a comparative study, MOG antibodies were shown to be common in patients with multiple sclerosis (Reindl (1999) Brain, 122, 2047-2056). Therefore, and due to the central role of MOG-specific B-cells in MS in a further embodiment of the present invention, the composition of the present invention is particularly useful for the selective elimination of these autoreactive lymphocytes (see herein below).

Cytochromes P450 (CYPs) and UDP-glucuronosyltransferases (UGTs) are targets of autoantibodies in several hepatic and extrahepatic autoimmune diseases (Obermayer-Straup (2000) Can.J Gastroenterol 14, 429-39) .

A number of Golgi proteins have been described as autoantigens, recognized by sera from patients with various forms of rheumatic diseases. Examples of Golgi autoantigens are golgin-67, golgin-95/gm130, golgin-160, giantin, golgin-97, p230, golgin245 and p210 (Eystathioy, (2000) J Autoimmun 14, 179-187; Mancini, (2000) J Cell Biol 149, 603-12, Linstedt (1993) Mol Biol Cell 4, 679-93; Griffith (1997) Arthritis Rheum 40, 1693-1702; Erlich (1996) J Biol Chem 271, 18328-18337; Fritzler (1995) J Biol Chem 270, 31262-31268; Fritzler (1993) J Exp Med. 178, 49-62; Fritzler (1984) J Immunol. 132, 2904-2908; Rios (1994) J Cell Biol 125, 997-1013; Renier (1994) J Autoimmun 7, 133-143).

In yet a further embodiment, the present invention provides for a composition for the selective elimination of autoreactive B-cells as described herein above, wherein said proteins sharing sequence similarity with microbial antigens or dietary proteins/components are selected from the group consisting of antigens mimicking proteins, polypeptides and/or carbohydrate structures from Streptococcus, Klebsiella, Proteus, M. tuberculosis, adenovirus, poliovirus, measles virus, retrovirus, papilloma virus, gluten and/or butyrophilin.

Microbial antigens can share regions of amino acid sequences homology with mammalian proteins. Said microbial antigens can, therefore, elicit an autoimmune response, being an example of antigenic mimicry. The composition of the present invention is, inter alia, useful for treating, preventing and/or ameliorating autoimmune responses due to such an antigenic mimicry of microbial organisms. Examples of exogenous antigenic mimicry are known in the art and, inter alia, described in Paul, "Fundamental Immunology", Raven Press, 1989.

For example, acute rheumatic fever is caused by antibodies against streptococcal cell-wall antigens that cross-react with cardiac muscle and lead to arthritis, myocarditis and late scarring of heart valves (Khanna (1997) J. Autoimmun. 10, 99-106; Bronze (1993) J. Immunol. 151, 2820-2828; Quinn. (1998) Infect Immun 66, 4418-4424). As discussed herein above, antibodies directed against the Sa antigen (streptococcal antigen) have been described in rheumatoid arthritis (Depres (1994), loc. cit.).

Furthermore, cross-reactivities of autoimmune antibodies exist between HLA-B27 and some Klebsiella strains, in particular of Klebsiella pneumoniae (see, inter alia, Domínguez-Lopez (2000) J. Rheumatol. 27, 1453-1460). This cross-reactivities lead to ankylosing spondylitis.

Another example of antigenic mimicry which leads to autoimmune reactions, especially in rheumatoid arthritis, is the cross-reaction to HLA-DR antigens with proteins/(poly)peptides of Proteus mirabilis (see, eg. Ebringer (1992), Ann. Rheum. Dis. 51, 1245-6 or Ebringer (2000), J Med Microbiol 49, 305-11).

Furthermore, an autoimmune pathogenesis of atherosclerosis is described and a cross-reactivity with human heat shock protein 60 (hsp60), expressed by endothelial cells, is involved in said autoimmune disease (Wick (2000), Herz 25,87-90).

In addition, the measles virus P3 protein resembles the above described autoantigen MBP and may elucidate EAE.

In a further embodiment, the present invention provides for a composition as described herein above, when said dietary component is gluten or butyrophilin.

In context of the present invention, dietary components are nutrients that share structural or sequence similarity with mammalian and/or human proteins or post-

translational modifications of human proteins, like N- or O-linked glycans. Such dietary components can cause molecular mimicry and induce an autoimmune reaction. Examples of dietary components are gluten and the milk constituent butyrophilin. Celiac disease, also referred to as gluten sensitive enteropathy is characterized by IgA autoantibodies against anti-tissue transglutaminase and antiendomysial antibodies (EMA). (Lock, J Clin Pathol 52 (1999), 274-277; Rose, J Am Acad Derm 41 (1999) 957-961; Vitoria, J Pediatr Gastro Nutr 29 (1999), 571-574; Schuppan, Gastroent 119 (2000), 234-242). Butyrophilin shares sequence homology to MOG. Butyrophilin has been shown to modulate animal models of multiple sclerosis due to molecular mimicry with MOG (Steffertl, J Immunol 165 (2000), 2859-2865).

The present invention also relates to a composition wherein said protein of intercellular structures as described herein above is selected from the group consisting of desmoglein-1 (Dsy1), desmoglein-3 (Dsy3), desmocollin, desmoplakin, envoplakin, periplakin, BP180, BPAG-1, BPAG-2 and HD1/plectin.

Pemphigus vulgaris and pemphigus foliaceus are caused by antibodies against keratinocyte adhesion molecules desmoglein 3 (Dsg3) and desmoglein I (Dsg1), respectively (Amagai, (1991) Cell 67, 869-877; Allen(1993), J Invest Dermatol 100, 685-91).

Other desmosomal or hemi-desmosomal proteins have been implied in pemphigus-related autoimmune diseases, in addition to desmogleins, also antibodies against desmoplakin, desmocollin, envoplakin, periplakin have been reported (Gooptu, (1999) Br. J. Dermatol. 141, 882-886; Chorzelski (1999) J Am Acad Dermatol 41, 393-400; Anhalt (1999) J. Am. Acad. Dermatol. 5, 763-6). Additional auto antigens are bullous pemphigoid antigens 1 (BPAG 1) and 2 (BPAG 2), BP230 (Schmidt, (2000) Arch Dermatol 136, 174-178; Michelson (2000) J Histochem Cytochem 48, 535-544; Schuhmann (2000) Am J Pathol 156, 685-95; Dopp (2000) J. Am. Acad. Dermatol. 42, 577-583).

HD1/plectin, another member of the plakin family, has been described to be recognized by sera from PNP (paraneoplastic pemphigus) patients (Proby (1999), J. Invest. Derm. 112, 153-156)

In a yet more preferred embodiment, the present invention relates to a composition, as described herein wherein said T cells are cytotoxic T cells.

Furthermore, the present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragment(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes, wherein said effector molecule is a receptor-ligand or the Fc-part of an immunoglobulin. It is particularly preferred that said effector molecule specifically binds to a molecule of the CD3-receptor complex. It is even more preferred that said receptor-ligand is an antibody or (a) fragment(s) or derivative thereof or an aptamer.

In accordance with the present invention the term "antibody" relates to monoclonal or polyclonal antibodies. Polyclonal antibodies (antiserum) can be obtained according to conventional protocols. Antibody fragments or derivatives comprise $F(ab')_2$, Fab, Fv or scFv fragments; see, for example, Harlow and Lane, "Antibodies, A Laboratory Manual", CSH Press 1988, Cold Spring Harbor, NY. Furthermore, in accordance with the present invention, the derivatives of the antibodies can be produced by peptidomimetics. In the context of the present invention, the term "aptamer" comprises nucleic acid aptamers such as RNA, ssDNA (ss = single stranded), modified RNA, modified ssDNA or PNAs which bind a plurality of target sequences having a high specificity and affinity. Nucleic acid aptamers are well known in the art and, inter alia, described in Famulok, Curr. Op. Chem. Biol. 2 (1998), 320-327. The preparation of aptamers is well known in the art and may involve, inter alia, the use of combinatorial RNA libraries to identify binding sites (Gold, Ann. Rev. Biochem. 64 (1995), 763-797). Said other receptors may, for example, be derived from said antibody etc. by peptidomimetics.

In a particular preferred embodiment of the present invention, the above mentioned antibody derivative is a scFv directed against a molecule of the CD3 receptor complex.

In accordance with the present invention, the term "molecule of the CD3 receptor complex" comprises any invariable proteins and or fragments thereof, which comprise CD3 α , CD3 β , CD3 γ , CD3 δ , CD3 ϵ and CD3 ζ (CD3 α and CD3 β are also known as TCR α and TCR β). It is particularly preferred that said scFv is directed against the CD3 ϵ chain of the T-cell receptor complex. Single chain constructs comprising such a specificity are known in the art and, inter alia, described in Mack (1997), J. Immunol. 158, 3965-3970 or in the appended illustrative examples.

In a most preferred embodiment, the present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragment(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes, wherein said domain comprising an autoreactive antigen or (a) fragment thereof is MOG or (a) fragment(s) thereof and wherein said domain comprising an immunological effector molecule is an anti-CD3 receptor or an Fc-part of an immunoglobulin.

The compositions as disclosed herein are in particular useful for the elimination of autoreactive B-cells in vitro, in vivo and ex vivo. The appended examples illustrate that not only in vitro depletions but also ex vivo as well as in vivo depletions are envisaged with the composition of the present invention. In addition, it is illustrated that the present invention also provides for compositions which may be employed for the reduction of auto-reactive, antigen-specific immunoglobulins.

The above mentioned composition will be particularly useful in treating, preventing and/or ameliorating autoimmune diseases, like multiple sclerosis. As described herein above, MOG is one of the major autoantigens involved in multiple sclerosis.

The protein and/or nucleotides sequence of MOG are known to the person skilled in the art and described, inter alia, in WO 95/06727 or US5532351. Approaches to affect the T cell arm of multiple sclerosis include induction of tolerance using synthetic peptides against the activation of MOG-specific T-helper cells (see, WO 95/07096, WO 96/12737, WO 97/35879, WO 99/12966). However, these approaches do not influence, ameliorate and/or modify the B-cell related symptoms of the autoimmune reaction in multiple sclerosis.

The above described MOG belongs to the family of B7 homologous proteins, sharing the membrane topology and the extracellular immunoglobulin domain (Johns, (1999) J. Neurochem. 72, 1-9). Several human homologues to MOG have been identified, but none of them has been reported to be an auto-antigen in MS. One homologue to MOG has been described that is expressed in B lymphocytes (WO 98/33912). Furthermore, fusion proteins of MOG or MOG homologues with Fc portions have been described (WO 98/33912, WO 99/23867), yet, said Fc-portion fusion proteins have been designed to enhance protein expression and for use as an affinity tag for purification. Often a protease site is introduced to remove the Fc portion after purification. This approach has been described in (EP 0 464 533). The above recited approaches of MOG-Fc fusion proteins have neither been proposed nor envisaged the uses and compositions described herein, namely for the elimination of autoreactive B-cells via binding, inter alia, to the B cell receptor and interaction with, e.g. complement or an Fc-receptor bearing effector cell.

The present invention relates in a further embodiment to a composition for the selective elimination of autoreactive B-cells wherein said at least one (poly) peptide construct is encoded by (a) a polynucleotide comprising a nucleic acid molecule encoding the polypeptide as depicted in SEQ ID NO. 2 or 4; (b) a polynucleotide comprising a nucleic acid molecule having the DNA sequence as depicted in SEQ ID NO. 1 or 3; (c) a polynucleotide hybridizing to a sequence which is complementary to a nucleotide sequence of (a) or (b); or (d) a nucleotide sequence being degenerate to the sequence of the nucleotide sequence of (c).

The above mentioned polynucleotide may be a naturally occurring nucleic acid

molecule as well as a recombinant nucleic acid molecule. Said polynucleotide/nucleic acid molecule may, therefore, be of natural origin, synthetic or semi-synthetic.

It is also immediately evident to the person skilled in the art that regulatory sequences may be added to the nucleic acid molecule of the invention. For example, promoters, transcriptional enhancers and/or sequences which allow for induced expression of the above described polynucleotide may be employed. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62).

The above described polynucleotide/nucleic acid molecules may either be DNA or RNA or a hybrid thereof.

With respect to the polynucleotides/nucleotide sequences characterized under (c) above, the term "hybridizing" in this context is understood as referring to conventional hybridization conditions, preferably such as hybridization in 50%formamide/6xSSC/0.1%SDS and 100µg/ml ssDNA, in which temperatures for hybridization are above 37°C and temperatures for washing in 0.1xSSC/0.1%SDS are above 55°C. Most preferably, the term "hybridizing" refers to stringent hybridization conditions, for example such as described in Sambrook., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

Furthermore, said polynucleotide/nucleic acid molecule may contain, for example, thioester bonds and/or nucleotide analogues. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell.

The polynucleotide/nucleic acid molecule of the composition of the present invention may be a recombinantly produced chimeric nucleic acid molecule comprising any of

the aforementioned nucleic acid molecules either alone or in combination. Preferably, the nucleic acid molecule of the invention is part of a vector.

In a particularly preferred embodiment, (poly)peptide construct of the composition of the present invention is a (poly)peptide comprising the amino acid sequence as depicted in SEQ ID NO. 2 or 4. Furthermore, (poly)peptide constructs are envisaged, wherein the sequence as shown in SEQ ID No.: 2 or 4 comprises at least one modification.

Said modification(s) may be selected from the group consisting of amino acid exchange(s), insertion(s), deletion(s), addition(s), substitution(s), inversion(s) and duplication(s). Said modification(s) also comprise conservative and/or homologue amino acid exchange(s). For example, guidance concerning how to make phenotypically/functionally silent amino acid substitution is given in Bowie (1990), Science 247, 1306-1310. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln; replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acid substitution, variants of peptides of this invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature (poly)peptide construct with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the (poly)peptide construct with additional amino acids, or leader or secretory sequence, or a sequence facilitating purification. Such variant (poly)peptide construct are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the present invention also relates to peptides which are at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably 90%, more preferably at least 95% and most preferably 99% identical or homologous to the (poly)peptide construct as shown in SEQ ID NO: 2 or 4. Specific strategies for

obtaining (poly)peptide constructs described herein above are known in the art. These methods comprise recombinant and biochemical methods and are, inter alia, disclosed in Sambrook, loc. cit.. Said methods also comprise protein engineering or direct synthesis.

Yet, in a further embodiment, the present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one polynucleotide encoding at least one (poly)peptide construct as described herein. The embodiments described herein above may be applied, mutatis mutantis, for the polynucleotide/nucleic acid molecule encoding said at least one (poly)peptide construct. It is understood that compositions as described herein and comprising at least one polynucleotide encoding at least one (poly)peptide construct as described herein may also be employed for the selective reduction of autoreactive, antigen-specific immunoglobulins.

Additionally, in a more preferred embodiment of the composition of the invention said polynucleotide/nucleic acid molecule is part of a vector. In a particularly preferred embodiment said vector is an expression vector.

Said vector of the present invention may be, e.g., a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions. Particularly preferred vectors are vectors as, inter alia, described in the appended examples and comprise, e.g. the expression vector CD19xCD3 pEF-dhfr.

Furthermore, the vector of the composition of the present invention, may in addition to the polynucleotides/nucleic acid sequences described herein above, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known to the artisan and may include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector. Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

Control elements ensuring expression in eukaryotic and prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in for example mammalian host cells comprise the CMV- HSV thymidine kinase promoter, SV40, RSV-promoter (Rous sarcoma virus), human elongation factor 1 α -promoter, enhancers, like CMV enhancer or SV40-enhancer. For the expression in prokaryotic cells, a multitude of promoters including, for example, the tac-lac-promoter or the trp promoter, has been described. Besides elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), pSPORT1, pEF-dhfr or prokaryotic expression vectors, such as lambda gt11, pDS or pET. Beside the nucleic acid described herein, the vector may further comprise nucleic acid sequences encoding for secretion signals. Such sequences are well known to the person skilled in the art. Furthermore, depending on the expression system used leader sequences capable of directing the peptides of the invention to a cellular compartment may be added to the coding sequence of the nucleic acid molecules of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a protein thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusionprotein including an C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the peptide(s) or fragments thereof of the invention may follow.

As mentioned herein above, the vector of the composition of the present invention may also be an expression vector. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Suitable vectors, methods or gene-delivering systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, *Nature Medicine* 2 (1996), 534-539; Schaper, *Circ. Res.* 79 (1996), 911-919; Anderson, *Science* 256 (1992), 808-813; Isner, *Lancet* 348 (1996), 370-374; Muhlhauser, *Circ. Res.* 77 (1995), 1077-1086; Onodua, *Blood* 91 (1998), 30-36; Verzeletti, *Hum. Gene Ther.* 9 (1998), 2243-2251; Verma, *Nature* 389 (1997), 239-242; Anderson, *Nature* 392 (Supp. 1998), 25-30; Wang, *Gene Therapy* 4 (1997), 393-400; Wang, *Nature Medicine* 2 (1996), 714-716; WO 94/29469; WO 97/00957; US 5,580,859; US 5,589,466; US 4,394,448 or Schaper, *Current Opinion in Biotechnology* 7 (1996), 635-640, and references cited therein. In particular, vectors and/or gene delivery systems are also described in gene therapy approaches in immunology or in neurology, for example Linden, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996), 11288-11294; Maass, *Hum. Gene Ther.* 9 (1998), 1049-1059; Hallek, *Cytokines Mol. Ther.* 2 (1996), 69-79; Peel, *Neurosci. Methods* 98 (2000), 95-104; Chen, *J. Neurosci. Res.* 55 (1999), 504-513. The nucleic acid molecules and vectors described herein and comprised in the composition of the present invention may be designed for direct introduction or for introduction via liposomes, viral vectors (e.g. adenoviral, retroviral), electroporation, ballistic (e.g. gene gun) or other delivery systems into the cell. Additionally, a baculoviral system can be used as eukaryotic expression system for the nucleic acid molecules of the invention. As documented in the appended examples, (poly) peptide constructs comprised in the composition of the present invention may also be expressed in mammalian expression systems, for example in CHO-cells.

As will be discussed herein below, the composition of the present invention comprising nucleic acid molecule as described herein above and/or the above described vectors/hosts may be particularly useful in treating, preventing and/or ameliorating an autoimmune disease/disorder. Therefore, said compositions may be employed in gene therapy approaches.

For gene therapy applications, said nucleic acids encoding the (poly)peptide constructs as described herein may be cloned into a gene delivering system, such as a virus

Therefore, it is particularly preferred that the of the present invention comprises a host transformed with the vector described herein above. It is even more preferred that said host is a mammalian cell, most preferred is a human cell.

In yet a further embodiment, the present invention relates to a composition as described herein above which further comprises a compound capable of selectively eliminating plasma cells and/or a compound capable of selectively eliminating (an) auto-antibody(ies). Preferably, said compound capable of selectively eliminating plasma cells is an antibody or (a) fragment(s) or a derivative thereof specifically detecting an plasma cell-specific epitope. Even more preferably, said compound capable of selectively eliminating (an) auto-antibody(ies) is an anti-idiotypic antibody or (a) fragment(s) or a derivative thereof specifically reacting with said auto-antibody(ies).

Furthermore, the present invention provides for compositions as described herein for the selective reduction of autoreactive immunoglobulins/for the selective elimination of auto-antibody(ies). As documented in the appended examples the constructs as disclosed herein and employed in the compositions of the present invention are not only capable of selectively eliminating autoreactive B-cells but also of reducing titers of autoreactive immunoglobulins. Preferably, said selective reduction of autoreactive immunoglobulins leads to a titer-reduction of at least 20%, at least 40%, at least 50%, at least 60%, most preferably at least 70%. Titers of autoreactive immunoglobulins may be measured by methods known in the art and as shown in the appended examples, e.g. the measurement of circulating autoreactive immunoglobulins. Such methods, e.g., methods of detecting autoantibodies in sera comprise ELISA-tests, RIA, immunodiffusions, immunoprecipitations, Western blotting, affinity-chromatographie. Furthermore, in situ-methods are envisaged which comprise the use of labeled peptide, preferably immunogold-labeled peptides and high-resolution microscopy.

In a most preferred embodiment of the present invention, the composition of the present invention is a pharmaceutical composition optionally comprising a pharmaceutically acceptable carrier.

The pharmaceutical composition of the present invention may be particularly useful in preventing, ameliorating and/or treating autoimmune disorders/diseases, as described herein above and herein below.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. It is particularly preferred that said administration is carried out by injection and/or delivery, e.g., to a site in a brain artery or directly into brain tissue. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an external or internal target site, like the brain. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it should also be in the range of 1 μ g to 10 mg units per kilogram of body weight per minute, respectively. In context of the present invention, it is preferred that the peptides of the present invention are employed in concentrations of less than 500 μ g/ml, more

preferred at less than 100 µg/ml, more preferred of less than 10 µg/ml and most preferred of less than 1 µg/ml.

Progress can be monitored by periodic assessment. The compositions of the invention may be administered locally or systemically. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition. Said agents may be drugs acting on the immune system, like FK506, cyclosporin, IFNbeta, azathioprine, cyclophosphamide, prednisone, corticosteroids, cyclosporin A, calcineurin, rapamycin and neuroprotective agents such as the neurotrophins (NGF, BDNF, NT-3); see also Webster, *Mult. Scler.* 3 (1997), 113-120; Ebadi, *Neurochem. Int.* 30 (1997), 347-374.

The compositions, also the pharmaceutical compositions, of the present invention may be tested for functionality by different approaches. For example, as a surrogate test system several approaches can be chosen: 1) Establishment of a B-cell derived cell line expressing a membrane-bound Ig derived from a murine hybridoma cell line specific for the auto-antigen, 2) Hybridoma cells expressing membrane-bound Ig could be isolated using auto-antigen; 3) Alternatively, autoreactive B cells may be derived from transgenic mice with autoreactive B cells. Mouse models which may be employed are known in the art. For example, Litzenburger (see *J. Exp. Med.* (1998) 188, 169-180) has established such a mouse model for multiple sclerosis using the DNA sequences of the heavy chain variable domain encoding the 8.18-C5 antibody to MOG. Further in-vivo models for B-cell dominant autoimmune diseases have been

reviewed in Murakami and Tasuku (Curr. Opin. Immunol. (1997) 9, 846-850), Christadoss et al. (Clin. Immunol. (2000) 94, 75-87). Experimental autoimmune encephalomyelitis (EAE) in rats and marmosets can be used as a surrogate model for multiple sclerosis ('t Hart et al. (2000) Immunol. Today 21, 290-297; Stefferl et al. (1999) J. Immunol. 163, 40-49). It is also envisaged that the above mentioned functionality approach comprises the measurement of circulating immunoglobulins and in particular of autoreactive immunoglobulins after administration of a composition as defined herein. Such a measurement is shown in the appended examples and may easily be employed in samples from test animals, as well as in samples of humans. Preferably, said sample is a blood sample.

The present invention relates in another embodiment to the use of at least one (poly)peptide construct as defined herein, of at least one polynucleotide described herein above or encoding at least one (poly)peptide construct as defined herein or of at least one vector described herein above for the preparation of a pharmaceutical composition for the treatment, amelioration and/or prevention of an autoimmune disease, preferably of a human autoimmune disease. Said pharmaceutical composition may be useful for the autoimmune diseases and disorders mentioned herein above and are particularly useful for the treatment, prevention or amelioration of diseases selected from the group consisting of Pemphigus vulgaris, Bullous pemphigoid, Goodpasture's syndrome, autoimmune haemolytic anemia (AIHA), rheumatoid arthritis, Systemic Lupus erythematosus, Grave's disease (autoimmune hyperthyroidism), contact dermatitis, Myasthenia gravis, juvenile diabetes, Sjögren's syndrome, autoimmune thyroiditis, primary hypoadrenalism (Addison's disease), multiple sclerosis, thrombocytopenic purpura, pemphigous foliaceus, Morbus Wegener (granulomatosis) and celiac disease.

The present invention also provides for a method of therapy, amelioration and/or prevention of an autoimmune disease comprising the administration to a subject in need of such therapy and/or prevention an effective amount of at least one (poly)peptide construct as defined herein,, of at least one polynucleotide as defined herein, of at least one vector defined herein or of at least one host of described herein It is particularly preferred that siad method be employed for the treatment

and/or prevention of autoimmune disorders as described herein, like, but not limited to, Pemphigus vulgaris, Bullous pemphigoid, Goodpasture's syndrome, autoimmune haemolytic anemia (AIHA), rheumatoid arthritis, Systemic Lupus erythematosus, Grave's disease (autoimmune hyperthyroidism), contact dermatitis, Myasthenia gravis, juvenile diabetes, Sjögren's syndrome, autoimmune thyroiditis, primary hypoadrenalism (Addison's disease), multiple sclerosis thrombocytopenic purpura, pemphigous foliaceus, Morbus Wegener (granulomatosis) and celiac disease. It is particularly preferred that the subject to be treated by the method of the invention be a human subject.

The compositions, in particular the pharmaceutical compositions, uses and methods of the invention can be used for all kinds of diseases hitherto unknown as being related to or dependent on auto-antigens and/or the production of auto-antibodies. Said compositions, uses and methods of the invention may be desirably employed in humans, although animal treatment is also encompassed by the uses and methods described herein.

In accordance with this invention, the terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. Said effect may be prophylactic in terms of completely or partially preventing a disease, in particular, an autoimmune disease, or a symptom thereof and/or may be therapeutic in terms of completely or partially curing a disease, in particular, an autoimmune disease, and/or (an) adverse effect(s) attributed to said disease. The term "treatment" as used herein includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e. arresting its development; or (c) relieving the disease, i.e. causing regression of the disease.

The figures show:

Fig. 1 Overview of concept for the elimination of autoreactive B cells. Overview of elimination of autoreactive B cells using molecules comprising an autoantigen and

an effector molecule domain. Abbreviations are ECR: Effector cell receptor; ECRbp: Effector cell receptor binding protein; Ag: Antigen.

Fig. 2. Schematic representation of autoantigen – effector domain molecules.
A. MOGxCD3 arrangement. B. MOG-Fc arrangement.

Fig. 3. Expression and purification of MOGxCD3. MOGxCD3 was expressed in CHO cells and purified by cation exchange, followed by nickel chelate chromatography and gel filtration. (A) 5 Gel filtration peaks are shown, separated by SDS-PAGE and analyzed by Coomassie-blue staining. Peak number 5 contains monomeric MOGxCD3 protein of a molecular weight of approximately 47kDa. (B) Gel filtration peaks were separated on SDS-PAGE identical to A, transferred to a nitrocellulose membrane and incubated with an anti-MOG antibody (8.18-C5). Only peak 5 containing the 47kDa monomeric MOGxCD3 reacts with monoclonal anti-MOG antibody (8.18-C5). Higher molecular weight bands present in peaks 1 to 4 are not recognized by anti-MOG antibody.

Fig. 4. Expression and purification of MOG-Fc fusion protein. MOG-Fc fusion protein was expressed in CHO cells and purified by affinity chromatography using Protein A sepharose. Purified protein was separated by SDS-PAGE and analyzed by coomassie-blue staining (A) in the presence of the reducing agent DTT, or by Western blotting on nitrocellulose (B), followed by detection using a monoclonal antibody against MOG (8.18-C5). As expected, MOG-Fc fusion protein has a molecular weight of approximately 50kDa under reducing conditions (+DTT), but runs at a molecular weight of approximately 115kDa in the absence of DTT (-DTT), indicative for a dimer under non-reducing conditions. Detection in (B) was with an anti-Fc monoclonal antibody.

Fig. 5. Detection of MOG fusion protein in ELISA. ELISA plates were coated with anti-MOG antibody 8.18-C5 to capture the fusion proteins by their MOG domain. (A) Bound MOG-Fc fusion protein was detected with an AP-conjugated anti-human Fc antibody. (B) MOGxCD3 fusion protein was detected by an AP-labeled chicken versus singlechain Fv anti-CD3 polyclonal antibody.

Fig. 6. Binding of MOG-Fc fusion protein to B-cells of a transgenic mouse. Resting B-lymphocytes (CD43⁻) were isolated and incubated with MOG-Fc. Bound fusion protein was detected by a FITC-labeled anti-human Fc-specific antibody. Only a subpopulation of transgenic B-cells express MOG-reactive B-cell receptors.

Fig. 7. Binding of MOGxCD3 to human T-cells. MOGxCD3 was incubated with human CD3-positive PBMCs, and bound fusion protein was detected with monoclonal anti-MOG antibody followed by FITC-labeled anti-mouse IgG1 antibody. Labeled cells were subjected to FACS analysis.

Fig. 8. 8.18-C5 hybridoma cells show surface expression of IgG1. Hybridomas were cultivated in serum-free medium for 4-5 months, and cell-surface IgG1 expression was examined by FACS analysis (A) with FITC-labelled anti-IgG1 antibody and (B) with biotinylated rMOG (recombinant MOG extracellular domain), followed by FITC-labeled streptavidin.

Fig. 9. Cytotoxic activity of autoantigen-effector proteins. FACS-based cytotoxicity assay with MOGxCD3 fusion protein. 8.18-C5 target cells were incubated with human CD3⁺ PBMCs and serial dilutions of MOGxCD3 protein in RPMI/10% FCS. Incubation was performed for 16h at 37°C/5% CO₂. After incubation, target cells were labeled with anti-IgG1 antibody and analyzed for viability through propidium iodide staining. Viable murine IgG1(+) cells were counted and expressed as percentage of viable target cells in control: (A) MOGxCD3, (B) MOG-Fc fusion protein.

Fig. 10. Specificity of elimination of autoreactive B cells. MOGxCD3 cytotoxicities. Parameters shown represent: specific toxicity as described in Fig 9; to measure spontaneous lysis target and effector cells had been incubated separately for the duration of the assay and were combined just prior to analysis: (A) MOGxCD3, (B) MOG-Fc.

Fig. 11. Cytotoxicity of MOGxCD3 is inhibited by recombinant MOG. MOGxCD3 was used at 0.1 µg/ml in FACS-based cytotoxicity assay as described for Fig. 9. Recombinant, biotinylated MOG was added to obtain a final concentration of 20

µg/ml.

Fig. 12. MOGxCD3 binds to 8.18-C5 target cells. Binding was assessed with FITC-labeled anti-HIS antibody (Dianova) at 10 µg/ml.

Fig. 13. Cytotoxic activity of MOG x CD3 is dependent on effector cell to target ratio. Assay was performed as described for Fig. 9 with varying concentrations of effector cells and a constant number of 8.18-C5 target cells.

Fig. 14. Flow cytometric analysis of cell binding properties of MOG-Fc.

(A) Binding of MOG-Fc to B cells of anti-MOG transgenic mice. Splenocytes from wt (upper panel) and anti-MOG transgenic mice (lower panel) were prepared and incubated with MOG-Fc. MOG-Fc bound to IgM-positive cells was detected by FITC-labeled human Fcγ-specific antibody.

(B, C) Binding of MOG-Fc to Mac1- and CD5-positive mouse splenocytes. Single-cell suspensions of wt splenocytes were prepared. Cells were incubated with 50 µg/ml MOG-Fc protein (lower panel) or PBS (upper panel). Cell-bound MOG-Fc was detected with biotinylated, monoclonal anti-MOG antibody 8.18-C5, followed by counterstaining with streptavidin-FITC, and Mac1-phycoerythrin (PE) or CD5-PE.

(D) Binding of MOG-Fc to a murine macrophage cell line. The monocyte/macrophage cell line p388.D1 expressing murine Fc receptors was incubated with MOG-Fc. Bound MOG-Fc was detected via FITC-labeled anti-human Fcγ antibody by flow cytometry (left panel). Binding was quantitated and expressed as mean fluorescence intensity (MFI) (right panel).

Fig. 15. Specificity of MOG-Fc mediated cytotoxicity

A) Effect of non-specific human IgG1.

8.18-C5 target cells were incubated with human PBMCs and 10 µg/ml MOG-Fc protein in RPMI/10% FCS. Incubation was performed for 16 h at 37°C and 5% CO₂. After incubation, target cells were labeled with anti-murine IgG1 antibody (mIgG) and analyzed for viability through propidium iodide (PI) staining. Column 1: incubation of PBMCs and target cells in the absence of isotype control and MOG-Fc. Column 2:

the effect of a recombinant human IgG1 (isotype control). Column 3: the effect of MOG-Fc under identical assay conditions.

B) Effect of murine cell line expressing cell surface IgG1 of non-MOG specificity.

Mouse B cell line TIB-208 is expressing cell surface IgG1 of non-MOG specificity. Column 1: unspecific lysis of TIB-208 cells in the presence of human PBMC. Column 2: the effect of MOG-Fc (10 µg/ml) on the viability of TIB-208 cells. Column 3: the effect of MOG-Fc (10 µg/ml) on hybridoma line 8.18-C5 under identical assay conditions. Error bars indicate S.D. values of triplicates.

Fig. 16. Ex vivo depletion of B-cells from anti-MOG transgenic mice.

Single cell suspensions of spleens from anti-MOG transgenic mice were prepared. Splenocytes were cultured in the absence (dark columns) or presence (white columns) of MOG-Fc (10 µg/ml). After incubation, the B cell population was analyzed by flow cytometry using antibodies against the B cell markers IgM, IgD and CD19. The frequency of cell populations is expressed as percentage of total live cells within the lymphocyte gate. Error bars represent S.D. values of triplicates.

Fig. 17. In vivo depletion of MOG-reactive B cells by MOG-Fc.

Anti-MOG transgenic mice were left untreated (control) or treated i.p. with 100 µg of MOG-Fc protein on days 1 and 3.

(A) Flow cytometric analysis of B cells from MOG-Fc-treated anti-MOG transgenic mice.

Upper panel, the effect of MOG-Fc on IgM^a (allotype a)-positive, rMOG-reactive B cells. Lower panel, analysis of MOG-Fc binding to B220-positive B cells as detected by an anti-human Fcγ antibody. Peripheral blood lymphocytes (PBLs) were prepared 1 day post-treatment and the MOG^{high} B-cell population was analyzed as indicated. Five MOG-Fc-treated and three control animals were analyzed. Representative results are shown.

(B) The effect of MOG-Fc on the MOG^{high} B-cell population in anti-MOG transgenic mice.

FACS data from five animals were quantitated and were shown as percentage of total B220⁺ cells. Bars give S.D. values.

Fig. 18 In vivo depletion of anti-MOG reactive B cells in wildtype mice after cellular transfer

1.5×10^7 B cells derived from anti-MOG transgenic mice with BL/6 background were transferred intravenously into wt BL/6. Mice were divided into 3 groups (N=5 per group) and treated with MOG-Fc (100 μ g each treatment) intraperitoneally 1, 2 and 3 days post-transfer (treated), with human IgG1 isotype (isotype control) and PBS (PBS).

24h (A) and 72h (B) after the last treatment, peripheral blood was collected by tail bleeding and analyzed for anti-MOG reactive B cells via FACS staining using recombinant MOG-Fc and PE-conjugated anti-human Fc antibody (ICN).

One day post-treatment with MOG-Fc depletion of MOG-reactive B cells (Fig. 18A) was observed. This effect was more prominent three days post-treatment showing a depletion of autoreactive B-cells in the order of 70% (Fig. 18B).

Fig. 19 Reduction of MOG-specific IgG in wildtype mice after cellular transfer

1.5×10^7 B cells derived from anti-MOG transgenic mice with BL/6 background were transferred intravenously into wildtype BL/6. Mice were divided into 3 groups (N=5 per group) and treated with MOG-Fc, human IgG1 isotype control and PBS. Peripheral blood was collected by tail bleeding 24 h (Fig. 19A) and 72 h (Fig. 19B) post-treatment and serum was analyzed for anti-MOG specific IgG titers by ELISA.

The present invention is additionally described by way of the following illustrative non-limiting examples, that provide a better understanding of the present invention and of its many advantages.

Example 1: Expression vectors for autoantigen fragment - α CD3 and -Fc fusion proteins

Expression of the construct of interest is driven by the promotor of the human elongation factor alpha (Kufer; PNAS 92 (1995): 7021). This promotor is known to be very efficient in virtually all eukaryotic cells, thereby making this expression system a powerful tool for high protein expression without limitations regarding the selected eukaryotic host cell line. A versatile multiple cloning site (MCS) facilitates the

cloning of the construct. The expression of the construct of interest is linked to the expression of the selection marker dihydrofolate reductase (DHFR) via the internal ribosomal entry site (IRES). This arrangement assures that almost all stably transfected cells will express the construct, as both genes depend on the promoter of EF α . A strong polyadenylation signal for both genes is provided by the SV40 polyadenylation site, and the pUC18 backbone of the plasmid provides a well-characterized plasmid backbone with ampicillin resistance for bacterial selection.

Example 2: Construction of an exemplified antigen-effector molecule; An auto-antigen x α CD3 fusion protein: MOGxCD3

The expression vector pEF-dhfr CD19xCD3 (Löffler; Blood 95 (2000): 2098) was used as the basis for the construction of MOGxCD3 by removing the region coding for the single-chain α CD19 antibody. As described below, the remaining α CD3 expression cassette was used for insertion of cDNA coding for the auto-antigenic domain of the human MOG protein and provides an ideal system for further cloning of auto-antigen x α CD3 fusion proteins (Fig. 2A).

2.1. Isolation of RNA from MOG-transfected fibroblasts and cDNA synthesis

Total RNA was isolated from MOG-transfected fibroblasts (Schluesener, J. Immunol. 139 (1987), 4016-4021) using the Qiagen RNEasy RNA Extraction Kit according to the manufacturer's suggestions. RNA was dissolved in H₂O and stored at -80°C. cDNA was synthesized as follows: 2 μ g total RNA were added to 0.5 μ g Oligo-dT primer in a total reaction volume of 12 μ l. The reaction mixture was incubated at 70°C for 10 min. Then, 4 μ l 5x First Strand Buffer (Gibco BRL), 2 μ l 0.1M DTT and 1 μ l 10mM dNTPs were added. Incubation was performed at 42°C for 2 min, after which 200 U of Superscript II Reverse Transcriptase (RT) (Gibco BRL) were added. The reaction mixture was incubated for 50 min. at 42°C. Then, RT was inactivated due to a 15 min incubation step at 70°C. Isolated cDNA was stored at -20°C.

2.2. Amplification of MOG-coding cDNA fragments

The following primers were chosen to obtain the cDNA coding for the extracellular domain of the human MOG protein (MOG-ex):

Primer 1 (MOG-Ex 5'): 5'-TAGAATTCATGGCAAGCTTATCGAGACCC-3' (Seq ID No 5)

Primer 2 (MOG-Ex 3'): 5'-CATCCGGATCCAGGGCTCACCCAGTAGA-3' (Seq ID No 6)

Primers were designed to amplify the first 462 bases of the coding region for the leader sequence and extracellular domain of the human MOG protein. The primers contained EcoRI and BspEI restriction sites at 5' and 3' ends, respectively. Polymerase chain reactions (PCR) conditions were: 50 pmol primer, 1 µl dNTPs 10mM, 4 µl cDNA, 5 µl Pfu-buffer 5x (Stratagene) and 5 U Pfu-Polymerase (Stratagene) in a final volume of 50 µl. The final product of 474bp was verified on a ethidium bromide stained 2% agarose gel.

2.3 Construction of MOGxCD3 fusion protein

The expression vector CD19xCD3 pEF-dhfr was digested with EcoRI and BspEI, leading to removal of the fragment coding for the anti-CD19 scFv domain. The remaining vector-anti CD3 scFv portion was gel-extracted (gel extraction kit, Qiagen). Equally, MOG-ex was partially digested with BsaWI and EcoRI, and the corresponding fragment of 474bp of length isolated via gel-extraction. BsaWI restriction was chosen due to this enzyme's insensitivity to dam-methylation at the original BspEI restriction site. DNA was eluted in 30 µl Tris pH 8.5 and stored at -20°C. Ligation of isolated DNA fragments was performed with equal volumes of extracted DNA and 5 U of T4 DNA Ligase in a total volume of 20 µl in 1x T4 buffer (Roche Biochemicals) for 30 min. at room temperature (RT). Of each ligation reaction, 3 µl were used to transform E. coli XL-1 Blue as described above. Colonies were picked and subjected to MiniPrep analysis. Following analytical restriction enzyme digestion, appropriate clones were sequenced (Sequiseive, Munich). Clones were grown in 300 ml LB-Amp medium, and plasmid DNA was isolated using the Qiagen plasmid prep kit. Corresponding nucleotide and amino acid sequences of this construct are depicted and illustrated in SEQ ID NOs: 1 and 2.

Example 3: Construction of auto-antigen – Fc fusion protein: MOG-Fc**3.1. Isolation of RNA from HD69-transfected CHO cells and cDNA synthesis.**

Total RNA was isolated from HD69-transfected CHO cells (WO9846645) using the Qiagen RNEasy RNA Extraction Kit according to the manufacturer's suggestions. RNA was dissolved in H₂O and stored at –80°C. Complementary DNA synthesis was performed: briefly, 2 µg total RNA were added to 0.5 µg Oligo-dT primer in a total reaction volume of 12 µl. The reaction mixture was incubated at 70°C for 10 min. Then, 4 µl 5x First Strand Buffer (Gibco BRL), 2 µl 0.1M DTT and 1 µl 10mM dNTPs were added. Incubation was performed at 42°C for 2 min, after which 200 U of Superscript II Reverse Transcriptase (RT) (Gibco BRL) were added. The reaction mixture was incubated for 50 min. at 42°C. Then, RT was inactivated due to a 15 min incubation step at 70°C. Isolated cDNA was stored at –20°C.

3.2. Amplification of IgG1-coding cDNA fragments

In order to obtain cDNA coding for the Fc-domain of the human IgG1 antibody backbone, the following primers were chosen:

Primer 3 (IgG1-Fc 5'): 5'-TATCCGGAGAGCCCCACCTCTTGTGACAAAAC-3' (Seq ID No 7)

Primer 4 (IgG1-Fc 3'): 5'-GTGTCGACTCATTTACCCGGAGACAGGG-3' (Seq ID No 8)

Yet, an even more preferred primer (IgG1-Fc5') is the following:

Primer 5 (IgG1-Fc5'): 5'- TATCCGGAGAGCCCCAAATCTTGTGACAAAAC-3' (SEQ ID NO: 9)

Primers were designed to amplify the 699 bases coding for the Fc part of the human IgG1 backbone, while introducing BspEI and SalI restriction sites at 5' and 3' ends of the amplified fragment, respectively. Amplification was performed according to standard PCR protocols. Briefly, 50 pmol/ each of appropriate primer, 1 µl dNTPs 10mM, 4 µl cDNA, 5 µl Pfu-buffer 5x (Stratagene) and 5 U Pfu-Polymerase

(Stratagene) were added to a final volume of 50 μ l in H₂O. The final product, containing the flanking restriction sites, was 711bp in length.

DNA was recovered from PCR reaction mixture according to the manufacturer's suggestions (Boehringer High Pure PCR Product Purification Kit, cat. no. 1 732 676). Blunt-ended PCR products generated by Pfu DNA polymerase were ligated into pCR-script vector (Stratagene #211188) according to manufacturer's protocol (Stratagene). Plasmids were transformed into competent *E. coli* strain XL-1 Blue using 4 μ l of ligation product added to 50 μ l of *E. coli*. The mixture was incubated on ice for 10 min., 1 min. at 42°C, and then again on ice for 2 min. Thereafter, 150 μ l LB-medium were added and expression of ampicillin resistance genes was induced due to 45 min at 37°C while shaking. Reaction mixtures were plated on LB-Amp Agarose plates (50 μ g ampicillin/ ml) and incubated at 37°C for 16h. Colonies were picked and grown in LB-Amp medium (100 μ g/ ml) for 8-12h. Bacteria were spun down, and plasmid DNA was isolated according to manufacturer's suggestions (Plasmid Mini-Kit, Qiagen). DNA was subjected to restriction enzyme analysis, and suitable clones were sequenced (SequiServe, Munich). Correct clones were grown in 300 ml LB-Amp medium, and plasmid DNA was isolated according to manufacturer's instructions (PLasmid Maxi Kit, Qiagen).

3.3. Construction of MOG-Fc fusion protein

In order to obtain the desired construct (Fig 2B), expression vector CD19xCD3 pEF-dhfr was subjected to restriction with EcoRI and Sall, leading to removal of the fragment coding for CD19xCD3. The remaining linearized vector was gel-extracted (gel extraction kit, Qiagen). Equally, MOG-ex was partially digested with BsaWI and EcoRI, and the corresponding fragment of 474bp of length was isolated via gel-extraction as described above. BsaWI restriction was chosen due to this enzyme's insensitivity to dam-methylation at the original BspEI restriction site. DNA was eluted in 30 μ l Tris pH 8.5 and stored at -20°C. Ligation of isolated DNA fragments was performed with equal volumes of extracted DNA and 5 U of T4 DNA Ligase in a total volume of 20 μ l in 1x T4 buffer (Roche Biochemicals). Ligation was allowed to proceed for 30 min. at room temperature (RT). Of each ligation reaction, 3 μ l were

used to transform *E. coli* XL-1 Blue as described above. Colonies were picked and subjected to MiniPrep analysis. Following analytical restriction enzyme digestion, appropriate clones were sequenced (Sequserve, Munich). Correct clones were grown in 300 ml LB-Amp medium, and plasmid DNA was isolated using the Qiagen plasmid prep kit as described above.

The corresponding nucleotide and amino acid sequences of this specific, illustrative construct are depicted in SEQ ID NOs: 3 and 4, respectively.

Example 4: Expression and purification of MOGxCD3 fusion protein

4.1. Stable transfection of CHO cells

CHO cells were plated at 3×10^5 /well in tissue culture 6-well plates and incubated at 37°C overnight. 3 µg of DNA were pipetted in sterile Eppendorf tubes, supplemented with 100 µl MEM-α medium (Gibco BRL) and 10 µl SuperFect transfection reagent (Qiagen) and incubated for 10 min. at RT. 600 µl of MEM-α medium were added, and the reaction mixture was transferred to CHO cells. Following a 2h-incubation at 37°C, the supernatant was aspirated, cells were washed once with PBS, and 2 ml MEM-α medium (10% FCS, HT-supplement 1:100) were added to each well. Transfection efficiency was determined to be 10% via standard β-galactosidase control transfection. After 24h at 37°C, transfected cells were transferred to 10 ml cell culture bottles (Nuncclone Δ, Nalge Nunc International) and selected for expression of the dhfr vector via growth in non-supplemented MEM-α medium plus 10% dialysed FCS. Following 2 passages of confluent cells at 1:5 splitting ratios, transfectants were further selected by addition of 20 nM methotrexate (MTX) to the selection medium. Cells were passaged 3 times, whereafter MTX concentration was increased to 100 nM. Following a further 3 passages, MTX was added to a final concentration of 500 nM.

4.2. Purification of expressed MOGxCD3 fusion protein

Stably transfected CHO-cells were transferred to 500 ml roller-bottles (Nalge Nunc International) in 250 ml MEM-α, 500 nM MTX and 5% dialysed FCS. The following day, another volume of medium was added without FCS to obtain a final concentration of 2.5% FCS. Cells were grown for 1 day post confluency. Cells were

separated from the supernatant by centrifugation at 4500 rpm, 30 min. in a Rotanta 46 centrifuge, and recombinant protein was purified from cell culture supernatant in a three-step purification process including cation exchange chromatography, immobilized metal affinity chromatography (IMAC) and gel filtration.

GradiFrac System (Pharmacia) was used for chromatography. All chemicals were of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Cation exchange was performed on a HiTrap SP Sepharose column (Pharmacia) that was equilibrated with buffer A1 (20 mM MES pH 5.5). Cell culture supernatant was diluted 2:1 with buffer A1 and applied to the column (10ml) with a flow rate of 4 ml/min. Unbound sample was washed out with buffer A1 and the bound protein was eluted with 100% buffer B1 (20 mM MES pH 5.5, 1M NaCl). Eluted protein fractions were pooled for further purification.

IMAC was performed, using a HisTrap column (Pharmacia) that was loaded with NiSO₄ according to the manufacturer's protocol. The column was equilibrated with buffer A2 (20 mM NaPP pH 7.5, 0.4 M NaCl), and the sample was diluted 2:1 with buffer A2 to obtain a pH of 7. The sample was applied to the column (2 ml) with a flow rate of 1 ml/min and the column was washed with buffer A2 to remove unbound sample. Bound protein was eluted using a linear gradient of buffer B2 (20 mM NaPP pH 7.5, 0.4 M NaCl, 0.5 M Imidazol) (0-100% buffer B2 in 10 column volumes). Eluted protein fractions were pooled for further purification.

Gel filtration chromatography was performed on a Sephadex S200 HiPrep column (Pharmacia) equilibrated with PBS (Gibco). Eluted protein samples (flow rate 1 ml/min) were subjected to SDS-Page and western blotting for MOGxCD3 detection. The column was previously calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200).

Protein concentrations were determined using protein assay dye concentrate (BioRad) and IgG (Biorad) as standard protein.

SDS-PAGE under non-reducing conditions was performed with precast 4-12% Bis-Tris gels (NOVEX). Sample preparation and application were according to the manufacturer's protocol. The molecular weight was determined with SeeBlue protein standard (NOVEX). The gel was stained with colloidal Coomassie (NOVEX protocol; Fig 3A).

4.3. Western Blot

Fractions were analyzed by Western Blot and staining with anti-MOG antibody for the presence of MOGxCD3. Protein was blotted to reinforced nitrocellulose membrane (Optitran BA-S 83, Schleicher & Schuell) at 200 mA for 1h (blotting buffer: 48 mM Tris, 39 mM Glycin, 0.01 % SDS). Recombinant fusion protein was detected with anti-MOG monoclonal antibody (8.18C5) at 5 µg/ ml in PBS; bound anti-MOG ab was detected via anti-mouse IgG antibody, AP-conjugated at 1:10000 in PBS (Sigma A-2429). The membrane was stained with BCIP/NBT (Sigma B-5655). (Fig. 3B)

The purity of the isolated protein was >95% as determined by SDS-PAGE. The molecule had an apparent mass of 45 kDa consistent with the predicted size.

The final yield of purified protein was ca. 2.4 mg / l cell culture supernatant. The final product ran as an approximately 45 kDa protein under native conditions as determined by gel filtration in PBS. No higher molecular weight forms were detected, suggesting that MOGxCD3 is a monomer (see Fig. 3B).

Example 5: Expression and Purification of MOG-Fc Fusion Protein

5.1. CHO cells were transfected as described in Example 4.

Stably transfected CHO-cells were transferred to 500 ml roller-bottles (Nalge Nunc International) in 250 ml MEM- α , 500 nM MTX and 5% dialysed FCS. The following day, another volume of medium was added without FCS to obtain a final concentration of 2.5% FCS. Cells were grown for 1 day post confluency. Cells were separated from the supernatant by centrifugation at 4500 rpm, 30 min. in a Rotanta 46 centrifuge, and recombinant protein was purified using a 1-step purification procedure via Protein A affinity chromatography (HiTrap Protein A column, Pharmacia) on the GradiFrac System (Pharmacia). Column was equilibrated with 10

ml of buffer A (20 mM Tris pH 7.2), and 500 ml of cell culture supernatant were passed through the column. Flow rate was 2 ml/ min. Bound Protein was eluted with 20 mM citrate, pH 3, using a linear gradient. Fusion protein yield amounted to 10 mg/l. Protein was > 95% pure as assessed by Coomassie staining (Fig. 4A).

5.2. Protein Analysis MOG-Fc

MOG-Fc fusion protein was analyzed via SDS-PAGE as described for MOGxCD3. Coomassie Brilliant Blue staining under reducing conditions (Fig. 4A) revealed a major band at 50kDa, with a higher molecular weight lane at approximately 115kDa. Western blotting under reducing and non-reducing conditions was performed for further analysis (Fig. 4B); under reducing conditions (Fig. 4B, +DTT), MOG-Fc runs as a monomer of 50kDa, which corresponds to its predicted size. The native protein has a molecular mass of approximately 115kDa (Fig. 4B, -DTT) under non-reducing conditions. This suggests that MOG-Fc is a disulfide-bridge linked dimer in its native state, presumably cross-linked via two cysteine residues in the hinge-region of the human IgG1 part of the protein.

Example 6: Binding of auto-antigen fusion proteins to autoreactive B-cells

6.1. Source and isolation of autoreactive B-cells

Litzenburger et al., J. Exp. Med. 188 (1) (1998) 169-180) generated a transgenic mouse strain with an anti-MOG heavy chain variable region, derived from the anti-MOG mAb 8.18-C5 "knocked in" for the germline J_H locus. Such mice exclusively express the 8.18-C5 anti-MOG heavy chain, resulting in generation of approximately 30% MOG-reactivity in the B-cell pool, as assessed by binding to recombinant MOG. Whole lymphocytes from transgenic knock-in mice were prepared from spleen as described elsewhere (Iglesias J. Exp. Med. 188 (1): 169-180). Resting B-lymphocytes (CD43-) were isolated from whole lymphocyte preparations. Cells were suspended in 5 ml PBS, 1% BSA, and 150 µl biotinylated anti-CD43 ab (Pharmingen) were added. After 30 min. on ice, cells were washed twice with PBS/ 1% BSA, and streptavidin-conjugated Dynabeads were added to obtain a mean 3.3 beads/ cell. The mixture was incubated at 4°C for 30 min. while rotating, whereafter bound cells were separated magnetically.

6.2. FACS-based binding assay of auto-antigen fusion protein to autoreactive B-cells
Autoreactive B-cells were washed twice with FACS-buffer (PBS, 1% FCS, 0.05% NaN₃). Cells were incubated with 50 μ l fusion protein diluted to 1 and 10 μ g/ ml in FACS-buffer, respectively for 1h at 4°C. Bound MOG-Fc fusion protein was detected with goat- α IgG FITC/hu, Fc-specific (ICN 67-217) at 1:50 (Fig. 6), while bound MOGxCD3 was detected with FITC-labeled α -HIS 6 antibody (Dianova; Fig. 7).

Example 7: Binding of auto-antigen fusion protein to auto-antibody

7.1. Source of auto-antibodies

Hybridoma 8.18-C5 (Linington, MPI Neurobiology Martinsried) was cultivated in serum-free medium (Gibco). Cells were separated from supernatant by centrifugation, and mouse anti-MOG monoclonal IgG1 antibodies were purified using a 1-step purification procedure via Protein G affinity chromatography (HiTrap Protein G column, Pharmacia) on the GradiFrac System (Pharmacia). Column was equilibrated with 10 ml of buffer A (20 mM Tris pH 7.2), and 500 ml of cell culture supernatant were passed through the column. Flow rate was 2 ml/ min. Bound Protein was eluted with 20 mM citrate, pH 3, using a linear gradient. Antibody yield amounted to 4.5 mg/ l. Protein was > 95% pure as assessed by Coomassie staining.

7.2. Sandwich-ELISA for detection of MOG-Fc fusion protein

Isolated α MOG ab 8.18-C5 was used to detect purified MOG-Fc fusion protein and to verify existence of 1) functional extracellular domain of MOG protein and 2) Fc effector domain in the recombinant protein. MaxiSorp 96-well plates (Nalge Nunc International) were coated with α MOG at 5 μ g/ ml overnight at 4°C. Plates were blocked with 1% BSA for 1h at RT, washed with PBS/ 0.05% Tween 20. Plates were incubated with various dilutions of MOG-Fc fusion protein in PBS for 1h at RT, and bound fusion protein was detected using α -human IgG1 ab, Fc-specific and AP-conjugated (Sigma A-9544) at 1: 10,000. Alkaline phosphatase-conjugated antibody was stained with pNPP (Sigma N-2770) and quantitated on the SpectraFluor ELISA reader (Tecan).

7.3. Sandwich-ELISA for detection of MOGxCD3 fusion protein

Isolated α MOG ab 8.18-C5 was used to detect purified MOGxCD3 fusion protein and to verify existence of 1) functional extracellular domain of MOG protein and 2) anti-CD3 effector domain in the recombinant protein. MaxiSorp 96-well plates (Nalge Nunc International) were coated with α MOG at 5 μ g/ ml overnight at 4°C. Plates were blocked with 1% BSA for 1h at RT, washed with PBS/ 0.05% Tween 20. Plates were incubated with various dilutions of MOGxCD3 fusion protein in PBS for 1h at RT, and bound fusion protein was detected using chicken polyclonal serum against scFv anti-CD3 (Davids Biotechnology). Bound chicken Ab was detected using alkaline phosphatase (AP)-coupled donkey-a-chicken ab at 1:10000 (Dianova 703-055-155). Alkaline phosphatase-conjugated antibody was stained with pNPP (Sigma N-2770) and quantified on the SpectraFluor ELISA reader (Tecan, Fig 5B).

Example 8: Binding of auto-antigen fusion proteins to immune effector cells

8.1. Isolation of PBMCs

Buffy coats were diluted 1:2 in PBS and separated in Ficoll gradient of density 1.077 (Seromed Cat.No. L 6115). Lymphocytes were separated and washed twice with PBS. Erythrocytes were lysed with lysis buffer (8,29g NH₄Cl cell culture tested (Sigma A-0171),

1,0g KHCO₃ 0,037g EDTA, cell culture tested (Sigma E-6511);H₂O add. 1L). Thrombocytes were separated during 20 min of centrifugation at 100 x g. Remaining Lymphocytes were transferred to cell culture bottles and stored at 37°C/ 5% CO₂.

8.2. Isolation of CD3+ cells

Human T-cell enrichment columns (R&D Systems Cat. No. HTCC-500/525) were used for isolation of T-cells according to manufacturer's suggestions.

8.3. Binding assay of MOGxCD3 to CD3+ PBMCs

To explore the binding of MOGxCD3 fusion protein to CD3+ cells, 200,000 CD3+ cells were added to each well of a V-bottom microtiter plate (Greiner Laborftechnik). Recombinant MOGxCD3 protein was added to obtain final concentrations of 0.032 up to 100 μ g/ ml in a total volume of 50 μ l per well. Bound fusion protein was detected via binding of 8.18-C5 diluted 1:1000 in FACS-buffer, whereafter bound

mouse monoclonal antibody was stained with α -mouse IgG-FITC (Sigma F-6257) at 1:40 dilution (Fig. 6).

Example 9: Establishment of a Cytotoxicity Assay for MOGxCD3 and MOG-Fc fusion proteins

9.1. Establishment of cell-surface α MOG-positive hybridoma cell line

8.18-C5 hybridoma cell line (Schluesener, J. Immunol. 139 (1987), 4016-4021) was adapted to serum-free medium (Hybridoma SFM, Gibco). Cells were passaged 1:5 every third day, and cultured in 100% SFM for a period of 4-5 months. Thereafter, MOG-reactivity in the hybridoma pool was assessed by FACS-analysis, using biotinylated MOG protein for staining. Positive cells were identified and isolated individually in 96-well plates by FACS-sorting. Clones were expanded for a period of approximately 2 weeks, and MOG-reactivity was checked again as described above. Anti-MOG positive clones were identified, and those showing the greatest amount of MOG-reactivity were expanded and used as targets for in-vitro cytotoxicity assays. More than 90% of cells show cell-surface expression of mIgG1 (Fig. 8A) and bound rMOG (Fig. 8B).

9.2. Selective elimination of autoreactive B-cells

A FACS-based cytotoxicity assay was performed. Effector cells (500000), 8.18-C5 target cells and fusion protein were added in a total volume of 200 μ l RPMI/ 10% FCS to each well of a sterile round-bottom multititre plate (CoStar) and incubated overnight at 37°C. Target cells were added to obtain E:T-ratios of 10:1, and MOGxCD3/-Fc fusion protein was added to attain final concentrations of 0.1, 1 and 10 μ g/ ml. Cells were incubated for 16 h at 37°C, washed with FACS-buffer, and target cells were labeled with anti-murine IgG1 antibody (Sigma 6257) at 1:50 dilutions in FACS-buffer; incubation was performed at RT for 30 min. Dead cells were excluded by staining with propidium iodide, and cells were analyzed with a FACSCalibur (Becton Dickinson). Dose-dependent cytotoxicity is shown for MOGxCD3 in Fig. 9A and for MOG-Fc in Fig. 9B. Specific toxicity is shown in Fig. 10. assay conditions were as described above, unspecific toxicity was defined cytotoxicity measured in the absence of protein. Spontaneous lysis was determined by separately incubated target and effector cells in the absence of protein, the cells

were mixed prior to FACS analysis. The negative values reflect slight proliferation during the 16h incubation.

Example 10: Inhibition of MOGxCD3 cytotoxicity by recombinant MOG (rMOG)

The specificity of MOGxCD3 based cytotoxicity was tested by addition of soluble recombinant MOG. A FACS-based cytotoxicity assay was performed as described in example 9, using MOGxCD3 at a concentration of 0.1 µg/ml. Recombinant, biotinylated MOG (rMOG) was added to a final concentration of 20 µg/ml and the cytotoxicity assay performed for 16 h. As shown in Fig. 11, cytotoxic activity can be inhibited by the addition of rMOG.

Example 11: MOGxCD3 binds to 8.18-C5 target cells

Binding of MOGxCD3 to B cell carrying membrane Ig receptor specific for the MOG protein was tested by a FACS-based binding assay. 8.18-C5 target cells were incubated with purified MOGxCD3 fusion protein at various concentrations for 1 h at 4°C. Cells were washed twice with FACS-buffer, and bound fusion protein was detected through its C-terminal HIS-tag using FITC-labeled anti-HIS antibody (Dianova). Cells were analyzed by FACS scanning and mean fluorescence scores were calculated and are shown in Fig. 12. MOGxCD3 efficiently bound at concentrations below 5 µg/ml.

Example 12: Selective elimination of autoreactive B-cells at different effector-to-target ratios (E:T).

The dependency of MOGxCD3 cytotoxicity on the availability of effector cells was determined by a FACS-based cytotoxicity assay as described in example 9. Target cells were seeded at a constant density of 50000 cells/well, while the CD3+ human effector cell concentration was varied in order to obtain the desired E:T ratio. Fig. 13 shows the corresponding specific toxicity, demonstrating that MOGxCD3 cytotoxicity is dependent on effector cell number.

Example 13: Binding specificity of auto-antigen fusion proteins to autoreactive B-cells**13.1. Specific binding of MOG-Fc fusion protein to IgM+ B-cells derived from splenocytes from anti-MOG transgenic mice**

Binding of MOG-Fc as described in Example 3 and Fig. 4 to MOG-reactive B cells was investigated using splenocytes from anti-MOG transgenic mice (Fig. 14A, lower panel). In these mice, the endogenous heavy chain-J region has been replaced by the rearranged 8.18-C5 anti-MOG VDJ segment using knock-in technology (Litzenburger, J. Exp. Med. 188 (1998), 169-180). As a result, almost all B cells of transgenic mice express the MOG-specific heavy chain in combination with endogenous light chains.

Whole lymphocytes from transgenic knock-in mice were prepared from spleen as described elsewhere (Litzenburger, J. Exp. Med. 188 (1998), 169-180). Cells were incubated with MOG-Fc fusion protein for 20 min on ice, and bound MOG-Fc was detected with goat-anti-human IgG FITC antibody (ICN 67-217). It was shown in FACS analysis that >90% of all IgM-positive splenocytes from the anti-MOG transgenic mice (Fig. 14A, lower panel) but not of control littermate mice (Fig. 14A, upper panel) did bind recombinant MOG-Fc. Two populations of B-cells were observed: Approximately one third of IgM-positive B cells in anti-MOG transgenic mice bound MOG-Fc with high intensity while two thirds bound MOG-Fc with low intensity (Fig. 14A, lower panel). This binding pattern was reminiscent of the one reported using biotinylated, bacterially produced, recombinant rat MOG (rMOG) (Litzenburger, J. Exp. Med. 188 (1998), 169-180 and Litzenburger, J. Immunol. 165, (2000) 5360-5366). In this report, however, two thirds of the B cells derived from anti-MOG transgenic mice did not bind rMOG at all or only poorly, and only one third of B cells bound rMOG with a broad range of intensities. Such differences are likely due to an intrinsically higher affinity of these B cells to MOG-Fc which, in contrast to the bacterially generated rMOG, is dimeric, properly folded, N-glycosylated and has not undergone biotinylation.

13.2. Selective binding of MOG-Fc fusion protein to wildtype murine splenocytes

Binding of MOG-Fc (as described in Example 3 and Fig. 4) to wildtype splenocytes was also investigated. Single-cell suspensions of wildtype splenocytes were prepared. Cells were incubated with 50 µg/ml MOG-Fc protein or PBS.

In order to allow binding to low-affinity Fc receptors, incubation with MOG-Fc was performed with 10-times the concentration of protein as with splenocytes from anti-MOG transgenic mice (Litzenburger, J. Exp. Med. 188 (1998), 169-180) and for a period of 45 minutes at room temperature compared to 20 minutes on ice. The subsequent incubation with anti-Mac1, anti-CD5 or biotinylated 8.18-C5 antibodies was performed on ice for 20 min. As shown in Fig. 14B, MOG-Fc did bind to the Mac1^{high} (CD11b, BD Pharmingen) positive population, indicating a preferential binding to macrophages and myeloid CD8⁺ dendritic cells (DCs). The upper panels of Fig. 14B and C show incubations on ice alone without prior incubation with MOG-Fc at room temperature. No binding of MOG-Fc to CD5⁺ T cells could be detected emphasizing the selectivity for FcγR⁺ cells.

13.3. Specific binding of MOG-Fc fusion protein to murine macrophage cell line

The murine monocyte/macrophage cell line p388.D1 also bound MOG-Fc (as described in Example 3 and Fig. 4) as shown by FACS detection of MOG-Fc via FITC-labeled anti-human Fcγ antibody (Fig. 1D). Mean fluorescence intensity (MFI) values observed for MOG-Fc were similar to those obtained with an isotype control IgG1 (Raum, Cancer Immunol Immunother. 50, (2001), 141-150) (data not shown).

Example 14: Specificity of MOG-Fc mediated cytotoxicity

The specificity of cell lysis mediated by MOG-Fc (as described in Example 3 and Fig. 4) was investigated by using both unrelated human IgG1 (Raum, Cancer Immunol Immunother. 50, (2001), 141-150) and a mouse B cell line expressing unrelated IgG on its surface (TIB-208).

14.1. Effect of non-specific human IgG1

8.18-C5 target cells were incubated with human PBMCs and 10 µg/ml MOG-Fc protein (as described in Example 3 and Fig. 4) in RPMI with 10% FCS. Incubation was performed for 16 h at 37°C/5% CO₂. After incubation, target cells were detected

with FITC-labeled anti-murine IgG1 antibody (mIgG, Pharmingen) and analyzed for viability through propidium iodide (PI) staining. The EpCAM antigen-specific recombinant human control IgG1 (Raum, Cancer Immunol Immunother. 50, (2001), 141-150) can bind to human effector cells via its Fc part but not to MOG-specific immunoglobulin on hybridoma cells. This isotype control did not lead to significant redirected lysis of 8.18-C5 cells (Fig. 15A).

14.2. Effect of an unrelated murine cell line expressing cell surface IgG1

Mouse B cell line TIB-208 is expressing cell surface IgG1 of non-MOG specificity and was incubated with human PBMCs and 10 µg/ml MOG-Fc protein (as described in Example 3 and Fig. 4) in RPMI with 10% FCS. Incubation was performed for 16 h at 37°C and 5% CO₂. The mouse B cell line TIB-208 expressing cell-surface IgG of non-MOG specificity was not sensitive to MOG-Fc mediated cell lysis (Fig. 15B).

Example 15: MOG-Fc mediated depletion of splenocytes of anti-MOG transgenic mice ex vivo

To investigate the cytotoxic potential of MOG-Fc (as described in Example 3 and Fig. 4) on MOG-specific B cells in vivo, the fusion protein was tested in anti-MOG transgenic mice. Firstly, anti-MOG mice have an extremely high titer of circulating MOG-reactive antibodies (Litzenburger, J. Exp. Med., 188, (1998) 169-180) and secondly, almost all B cells in these mice do show MOG reactivity. MOG-Fc had the ability to deplete primary anti-MOG-positive B cells from anti-MOG transgenic mice ex vivo and in vivo. Elimination of MOG-reactive B cells ex vivo was efficient (on the order of 70% within 16 hours), while in vivo it was lower but still significant among the population of highly MOG-reactive B cells. MOG-reactive B cells are constantly replenished in the periphery by the bone marrow on the order of 10⁷ cells/day. In contrast, the frequency of autoreactive B cells in human autoimmune disease is extremely low (Link, J. Clin. Invest., 87, (1991), 2191-2195 and Nishifuji, J. Invest. Dermatol., 114, (2000), 88-94). Therefore, the anti-MOG transgenic mouse model represented the most difficult situation and highest possible hurdle to test the in-vivo efficacy of a specific B cell-eliminating protein such as MOG-Fc.

In order to assess whether MOG-Fc (as described in Example 3 and Fig. 4) can also eliminate normal B cells expressing MOG-reactive cell-surface immunoglobulin, splenocytes from anti-MOG transgenic mice were isolated and incubated with 10 µg/ml of MOG-Fc fusion protein for 16 h at 37°C and 5% CO₂ in DMEM with 10% FCS in 5 ml cell culture polypropylene vials (Becton-Dickinson, Pharmingen) at a density of 4x10⁶ cells/ml. Lymphocyte analysis was carried out by FACS using biotinylated recombinant MOG protein and antibodies against IgM^a, IgD and CD19 (all Becton-Dickinson, Pharmingen). All tests were carried out in triplicate. The endogenous Fcγ receptor-bearing cells served as effectors. In FACS analysis, a highly significant depletion of B cells positive for IgM^a, IgD and CD19 was observed (Fig. 16). The average degree of B cell depletion was on the order of 70% and was comparable for all three B-cell markers.

Example 16: In vivo depletion of anti-MOG specific B-cells in transgenic mice

The cytotoxic efficacy of MOG-Fc (as described in Example 3 and Fig. 4) was tested in vivo using the anti-MOG transgenic mouse. This mouse strain carries the anti-MOG heavy chain variable region, derived from the anti-MOG monoclonal antibody 8.18-C5 "knocked in" for the germline JH locus (Litzenburger, J. Exp. Med. 188 (1998), 169-180).

Female anti-MOG transgenic mice were treated twice with 100 µg of recombinant MOG-Fc fusion protein in 500 µl PBS through i.p. injection (n=5) on day 1 and 3. Blood was collected each day after treatment, PBLs were prepared, and lymphocytes were analyzed via FACS (FACSCalibur, Becton-Dickinson) analysis one day post treatment. MOG⁺ B cells were quantitated and normalized as percentage of total B220⁺ B cells detected in the lymphocyte gate. MOG-Fc was efficient in depleting IgM-positive B cells in the fraction that strongly reacted with MOG (Fig. 17A, upper panel). MOG-Fc was still bound to B cells 24 h post-treatment (Fig. 17B) as detected by staining of isolated B220-positive blood lymphocytes with anti-human Fcγ specific antibody.

The percentage of highly MOG expressing B-cells decreased with statistical significance from 45% in control (untreated mice) to less than 30% on day 4 after the first treatment (Fig. 17B). The number of B220⁺ B cells was decreased to the same extent as the number of IgM-positive B cells (data not shown), suggesting that the

reduction of B cells measured by flow cytometry was due to a depletion of cells and not to a down-modulation of the B-cell receptor expression.

Example 17: Depletion of autoreactive B cells and reduction of MOG-specific IgG in wildtype mice after cellular transfer

17.1 In vivo depletion of anti-MOG reactive B cells in wildtype mice after cellular transfer

In order to study the depleting potential of MOG-Fc on a limited number of B-cells derived from anti-MOG transgenic mice in vivo, 1.5×10^7 B cells derived from anti-MOG transgenic mice with BL/6 background were transferred intravenously into wt BL/6.

Wild-type mice transferred with a limited number of autoreactive B-cells can be used as a model system reflecting the situation in B-cell-mediated autoimmunity which is characterized by the presence of a limited number of autoreactive B-cells versus a high background of endogenous normal B lymphocytes. Thus, this system is very close to the real-life situation and confirms the data on depletion of MOG-reactive B cells obtained in the anti-MOG transgenic mice.

Mice were divided into 3 groups (N=5 per group). Group A was treated with MOG-Fc (100ug each treatment) intraperitoneally 1, 2 and 3 days post-transfer. Control groups were treated with human IgG1 isotype control (Raum, Cancer Immunol Immunother. 50, (2001), 141-150, group B) and PBS (group C). For this purpose splenocytes derived from anti-MOG transgenic mice with BL/6 background were prepared as single-cell suspension and stimulated with LPS as described (Litzenburger, J. Exp. Med. 188 (1998), 169-180). Three days post-stimulation at 37°C/ 5% CO₂, resting B-cells (CD43-) were isolated (Litzenburger, J. Exp. Med. 188 (1998), 169-180) and 1.5×10^7 cells were transferred intravenously into wildtype BL/6 mice on the same day. Mice were treated as described above on days 1,2 and 3 following transfer. 24h and 72h after the last treatment, peripheral blood was collected by tail bleeding and analyzed for anti-MOG reactive B cells via FACS staining (FACS Calibur, Becton-Dickinson) with recombinant MOG-Fc (as described in Example 3 and Fig. 4) followed by detection with PE-conjugated anti-human Fc antibody (ICN).

One day post-treatment with MOG-Fc depletion of MOG-reactive B cells (Fig. 18A) was observed. This effect was more prominent three days post-treatment showing a depletion of autoreactive B-cells in the order of 70% (Fig. 18B).

17.2. Reduction of MOG-specific IgG in wildtype mice after cellular transfer

1.5×10^7 B cells derived from anti-MOG transgenic mice with BL/6 background were transferred intravenously into wt BL/6 as described in Example 17.1. Mice were divided into 3 groups (N=5 per group) and treated with MOG-Fc (100ug each treatment) intraperitoneally 1, 2 and 3 days post-transfer (A), human IgG1 isotype control (Raum, Cancer Immunol Immunother. 50, (2001), 141-150, group B) and PBS (group C). Peripheral blood was collected by tail bleeding and sera from day 1 and 3 post-treatment were analyzed for anti-MOG specific IgG titers by ELISA. ELISA plates were coated with recombinant MOG (10 μ g/ml), serum samples collected 24 h post-treatment were applied at 1:100 dilution and serum samples collected at 72h post-treatment were applied at 1:1000 dilution in PBS. Bound MOG-reactive IgG was detected with biotinylated rat-anti-mouse IgG antibody (Jackson Laboratories). Streptavidin-AP and pNPP (Sigma N-2770) were used for detection and OD values were quantified on a Dynatech MR4000 ELISA reader.

MOG-Fc had the ability to reduce the number of circulating, MOG-reactive IgG after 24h (Fig. 19A) and 72h (Fig. 19B), presumably via a combination of direct adsorption and neutralization of MOG-reactive immunoglobulin.

Both effects described in Example 17.1. and 17.2. are of high therapeutic value, since immunoabsorption of autoreactive immunoglobulin leads to a short-term effect through depletion of pathogenic antibodies, while elimination of autoreactive B cells depletes the pathogenic cellular reservoir.

Claims

1. A composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragment(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes and/or capable of activating the complement system.
 2. The composition of claim 1, wherein said construct is a fusion (poly)peptide or a mosaic (poly)peptide.
 3. The composition of claim 1, wherein said construct is a cross-linked (poly)peptide construct.
 4. The composition of any one of claims 1 to 3, wherein said autoreactive antigen or (a) fragment(s) thereof is selected from the group consisting of intracellular matrix proteins, extracellular matrix proteins, complement factors, nuclear antigens, cell surface receptors, nuclear receptors, lipids, soluble factors, membrane proteins, heat shock proteins, proteins with sequence similarity to microbial antigens or to dietary components and proteins of intercellular structures.
 5. The composition of claim 4, wherein said intracellular matrix protein is selected from the group consisting of keratin, filaggrin, antiperinuclear factor
- 7.

6. The composition of claim 4, wherein said extracellular matrix protein is collagen.
7. The composition of claim 4, wherein said complement factor is C5.
8. The composition of claim 4, wherein said nuclear antigen is selected from the group consisting of DNA, histones, snRNPs, topoisomerase I, ro (SS-A-Ro), la (SS-B-La), Scl-70, centromer protein (CENP), large ribonuclear particles (RNPs), A2, Sm proteins, tRNA synthetase and Ku antigen.
9. The composition of claim 4, wherein said cell surface receptor is selected from the group consisting of TSH-receptor, Ach-receptor, asialo-glycoprotein receptor and platelet integrin GpIb:IIIa.
10. The composition of claim 4, wherein said soluble factor is selected from the group consisting of i-antigen, Rh-blood group factor, 21-hydrolase enzyme, glutamic acid decarboxylase (GAD), insulin, (ICA) 512, ICAP-69, (tissue) transglutaminase (tTG), transaldolase, S100beta, oxidized low-density lipoprotein (ox-LDL), CNPase, proteinase 3 and type I antigen.
11. The composition of claim 4, wherein said membrane protein is selected from the group consisting of PLP, MAG, MBP, MOG, Golgi-proteins, cytochrome P450 (CYPs) and UDP-glucuronosyltransferase (UGTs).
12. The composition of claim 4, wherein said heat shock protein is selected from the group consisting of alpha B-crystallin, Hsp27, HSP70 and HSP60.
13. The composition of claim 4, wherein said proteins with sequence similarity to microbial antigens or to dietary components are selected from the group consisting of antigens mimicking proteins, polypeptides and/or carbohydrate structures from Streptococcus, Klebsiella, Proteus, M. tuberculosis, adenovirus, poliovirus, retrovirus, papillomavirus, measles virus, gluten or butyrophilin.

14. The composition of claim 4, wherein said protein of intercellular structures is selected from the group consisting of desmoglein-1 (Dsy1), desmoglein-3 (Dsy3), desmocollin, desmoplakin, envoplakin, periplakin, BPAG-1, BPAG-2 and/or HD1/plektin.
15. The composition of any one of claims 1 to 14, wherein said T cells are cytotoxic T cells.
16. The composition of any one of claims 1 to 15, wherein said effector molecule is a receptor-ligand or the Fc-part of an immunoglobulin.
17. The composition of claim 16, wherein said effector molecule specifically binds to a molecule of the CD3-receptor complex.
18. The composition of claim 16 or 17, wherein said receptor-ligand is an antibody or (a) fragment(s) or derivative thereof or an aptamer.
19. The composition of claim 18, wherein said antibody derivative is a scFv directed against a molecule of the CD3 receptor complex.
20. The composition of any one of claims 1 to 4, wherein said domain comprising an autoreactive antigen or (a) fragment thereof is MOG or (a) fragment(s) thereof and wherein said domain comprising an immunological effector molecule is an anti-CD3 receptor or an Fc-part of an immunoglobulin.
21. The composition of claim 20, wherein said (poly) peptide construct is encoded by
 - (a) a polynucleotide comprising a nucleic acid molecule encoding the polypeptide as depicted in SEQ ID NO. 2 or 4;
 - (b) a polynucleotide comprising a nucleic acid molecule having the DNA sequence as depicted in SEQ ID NO. 1 or 3;

- (c) a nucleotide sequence hybridizing to a sequence which is complementary to a nucleotide sequence of (a) or (b); or
 - (d) a nucleotide sequence being degenerate to the sequence of the nucleotide sequence of (c).
22. The composition of claim 20, wherein said (poly)peptide construct is a (poly)peptide comprising the amino acid sequence as depicted in SEQ ID NO. 2 or 4
23. A composition for the selective elimination of autoreactive B-cells comprising a (poly) nucleotide encoding at least one (poly) peptide construct as defined in any one of claims 1 to 22.
24. A composition for the selective elimination of autoreactive B-cells comprising a vector comprising a (poly) nucleotide as defined in claim 23.
25. The composition of claim 24, wherein said vector is an expression vector.
26. A composition for the selective elimination of autoreactive B-cells comprising a host transformed with the vector of claim 24 or 25.
27. The composition of claim 26, wherein said host is a mammalian cell.
28. The composition of any one of claims 1 to 27 further comprising a compound capable of selectively eliminating plasma cells and/or a compound capable of selectively eliminating (an) auto-antibody(ies).
29. The composition of claim 28, wherein said compound capable of selectively eliminating plasma cells is an antibody or (a) fragment(s) or a derivative thereof specifically detecting an plasma cell-specific epitope.
30. The composition of claim 28, when said compound capable of selectively eliminating (an) auto-antibody(ies) is an anti-idiotypic antibody or (a)

fragment(s) or a derivative thereof specifically reacting with said auto-antibody(ies).

31. The composition of any one of claims 1 to 30 which is a pharmaceutical composition optionally comprising a pharmaceutically acceptable carrier.
32. The composition of any one of claims 1 to 31 for selective reduction of autoreactive immunoglobulins.
33. Use of at least one (poly)peptide construct as defined in any one of claims 1 to 31 for the preparation of a pharmaceutical composition for the treatment and/or prevention of an autoimmune disease.
34. Use of a polynucleotide encoding at least one (poly)peptide construct as defined in any one of claims 1 to 22 for the preparation of a pharmaceutical composition for the treatment and/or prevention of an autoimmune disease.
35. Use of the vector of claim 24 or 25 or of the host of claim 26 or 27 for the preparation of a pharmaceutical composition for the treatment and/or prevention of an autoimmune disease.
36. The use of any one of claims 33 to 35, wherein said autoimmune disease is selected from the group consisting of Pemphigus vulgaris, Bullous pemphigoid, Goodpasture's syndrome, autoimmune haemolytic anemia (AIHA), rheumatoid arthritis, Systemic Lupus erythematosus, Grave's disease (autoimmune hyperthyroidism), contact dermatitis, Myastenia gravis, juvenile diabetes, Sjögren's syndrome, autoimmune throiditis, primary hypoadrenalism (Addison's disease), multiple sclerosis, thrombocytopenic purpura, pemphigous foliaceous and celiac disease.
37. A method of therapy, amelioration and/or prevention of an autoimmune disease comprising the administration to a subject in need of such therapy and/or prevention an effective amount of at least one (poly)peptide construct

as defined in any one of claims 1 to 21, of at least one polynucleotide as defined in claim 20 or 21, of at least one vector as defined in claim 24 or 25 or of at least one host as defined in claim 26 or 27.

38. The method of claim 37, wherein said autoimmune disease is selected from the group consisting of Pemphigus vulgaris, Bullous pemphigoid, Goodpasture's syndrome, autoimmune haemolytic anemia (AIHA), rheumatoid arthritis, Systemic Lupus erythematosus, Grave's disease (autoimmune hyperthyroidism), contact dermatitis, Myasthenia gravis, juvenile diabetes, Sjögren's syndrome, autoimmune thyroiditis, primary hypoadrenalism (Addison's disease), multiple sclerosis thrombocytopenic purpura, Morbus Wegener (granulomatosis), pemphigous foliaceus and celiac disease.

Fig. 1

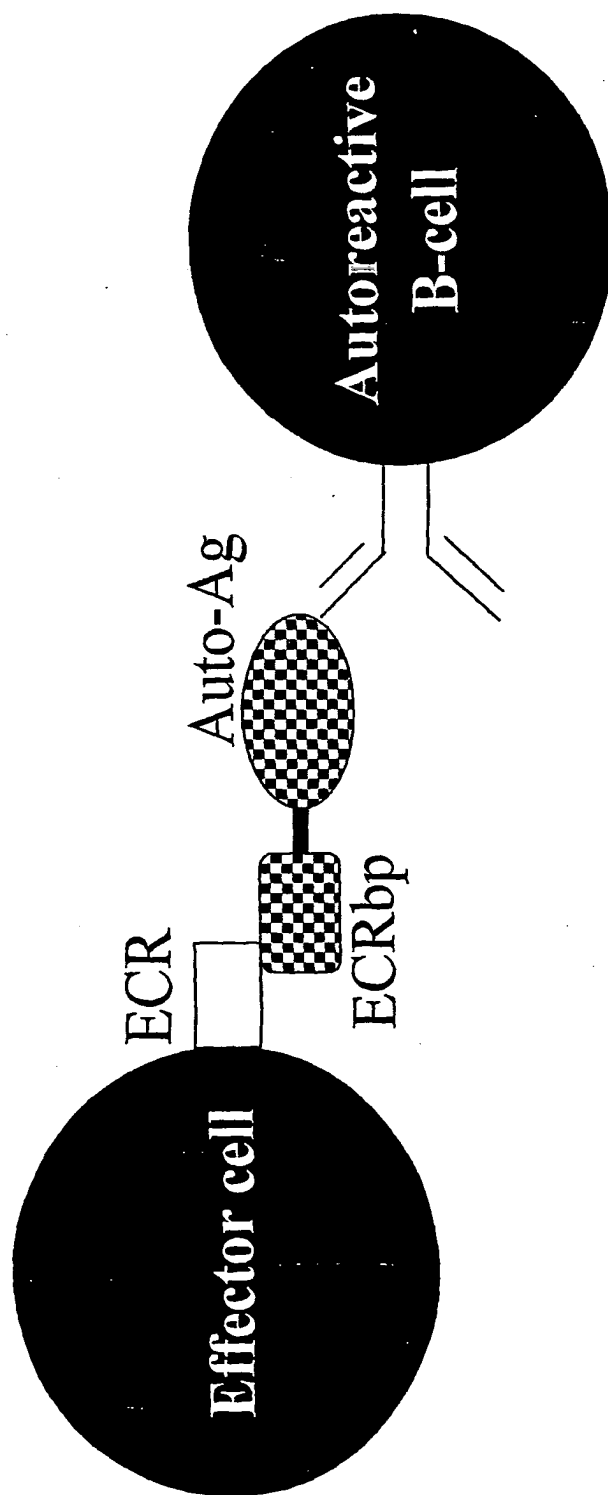


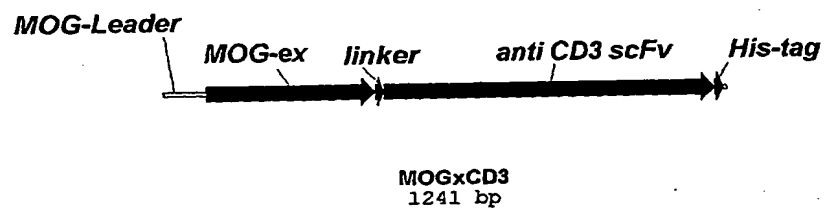
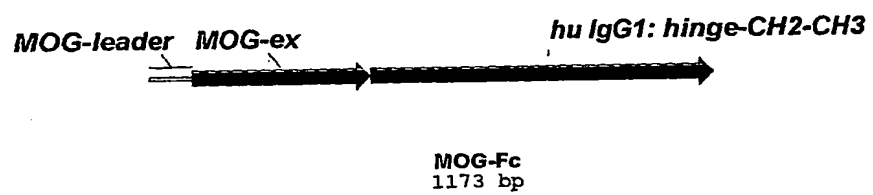
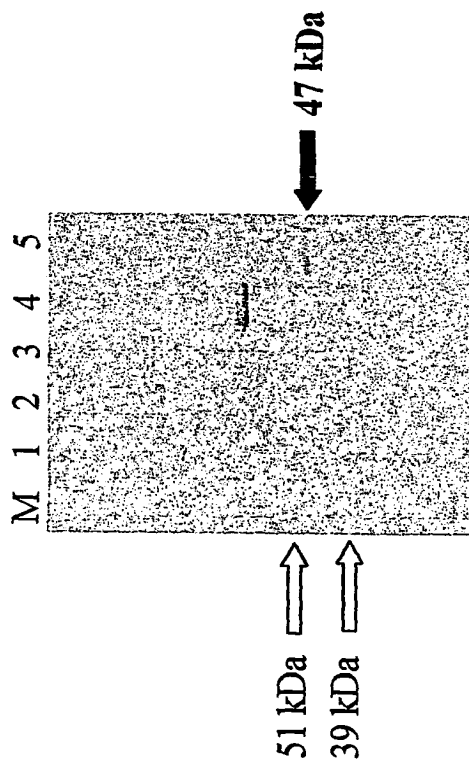
Fig. 2**A.****B.**

Fig. 3

A



B

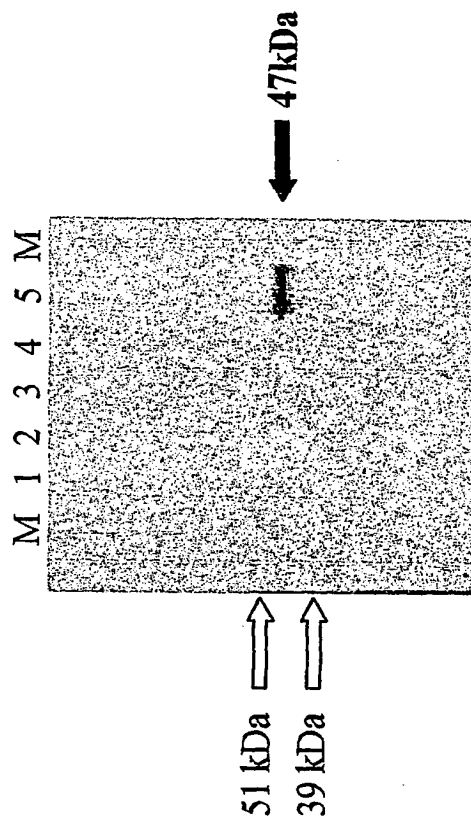


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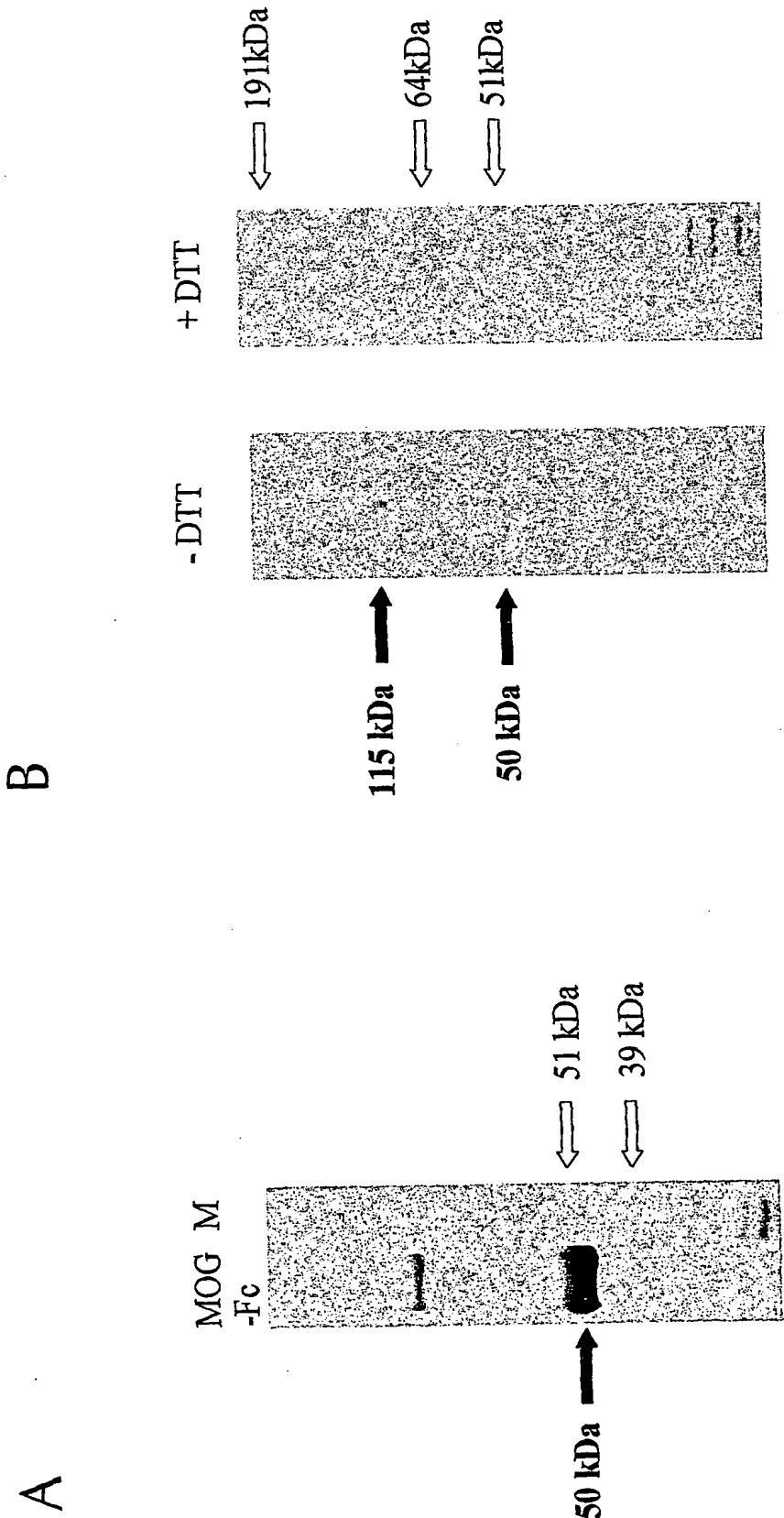


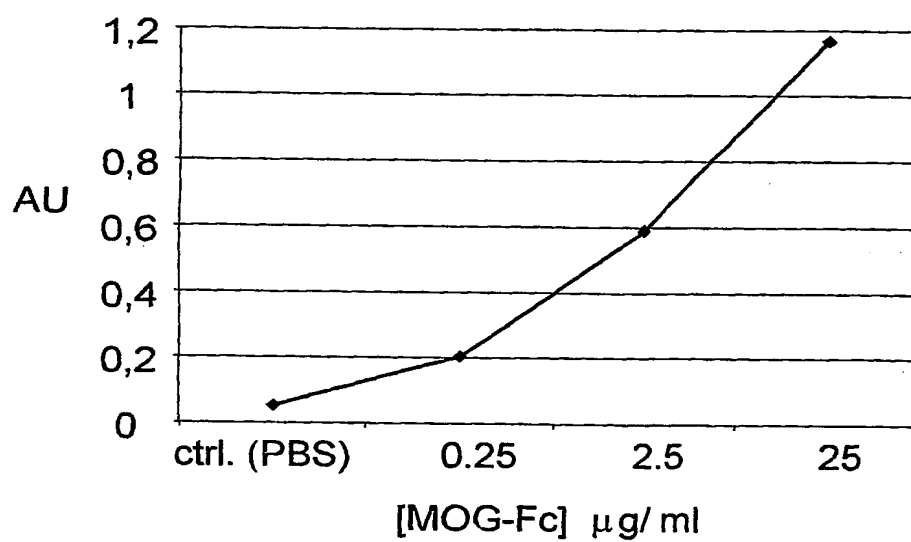
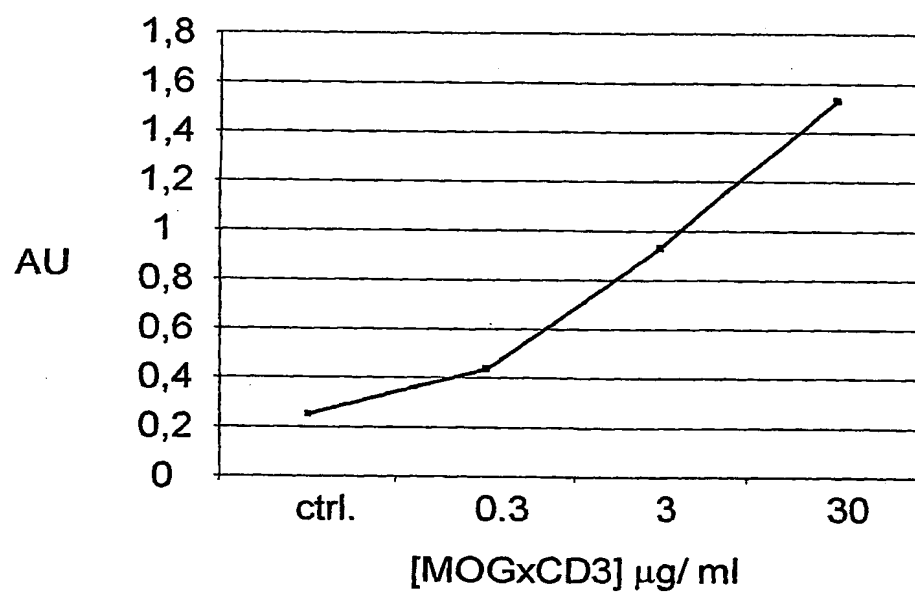
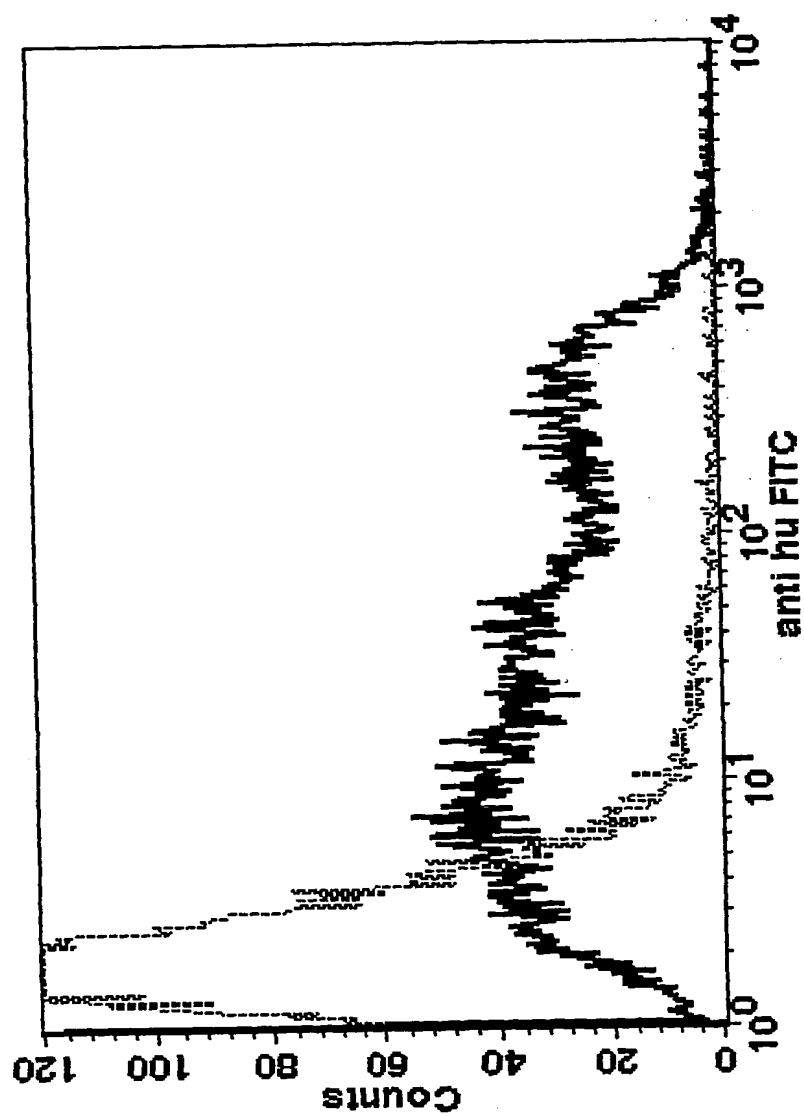
Fig. 5**A****B**

Fig. 6



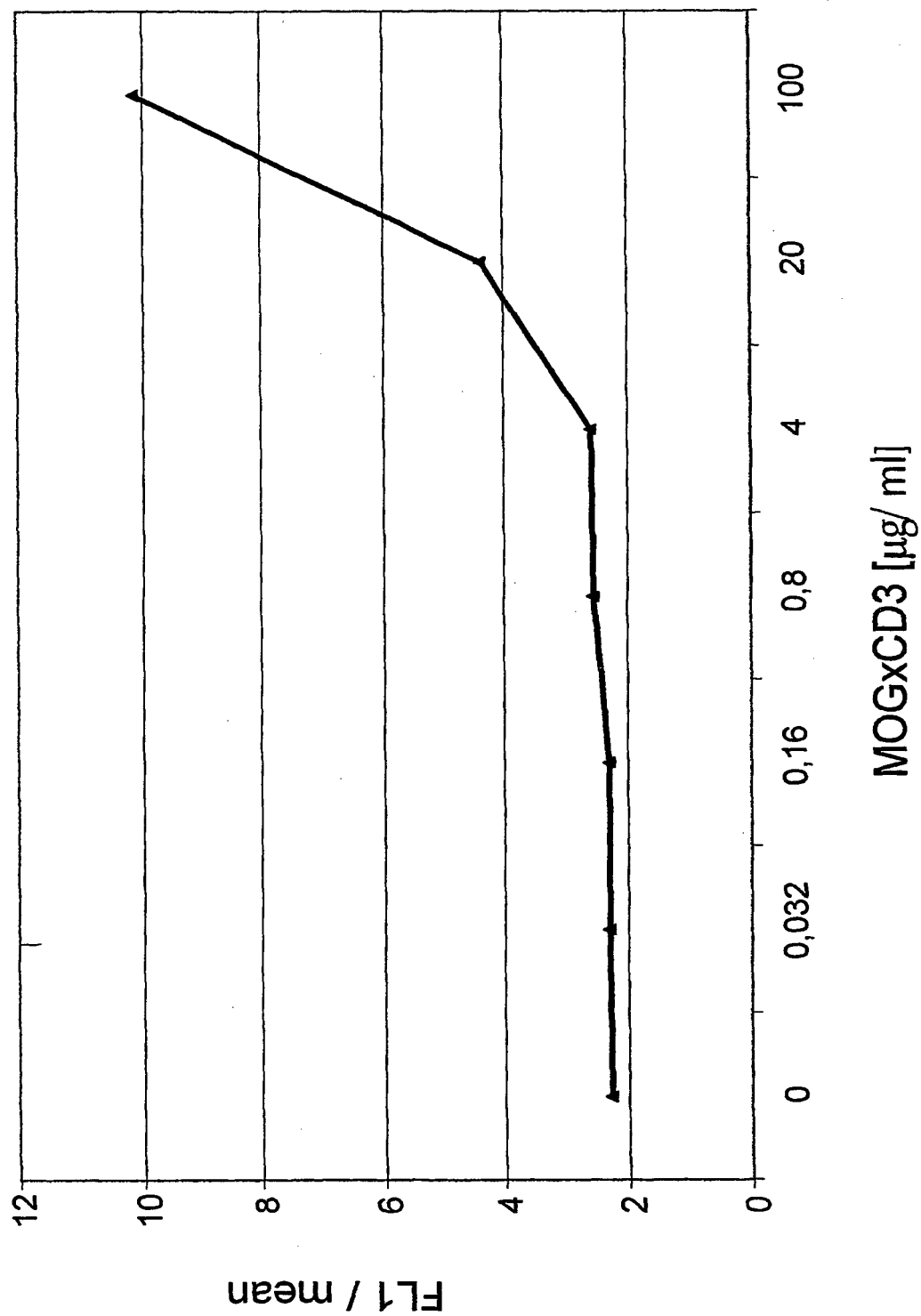


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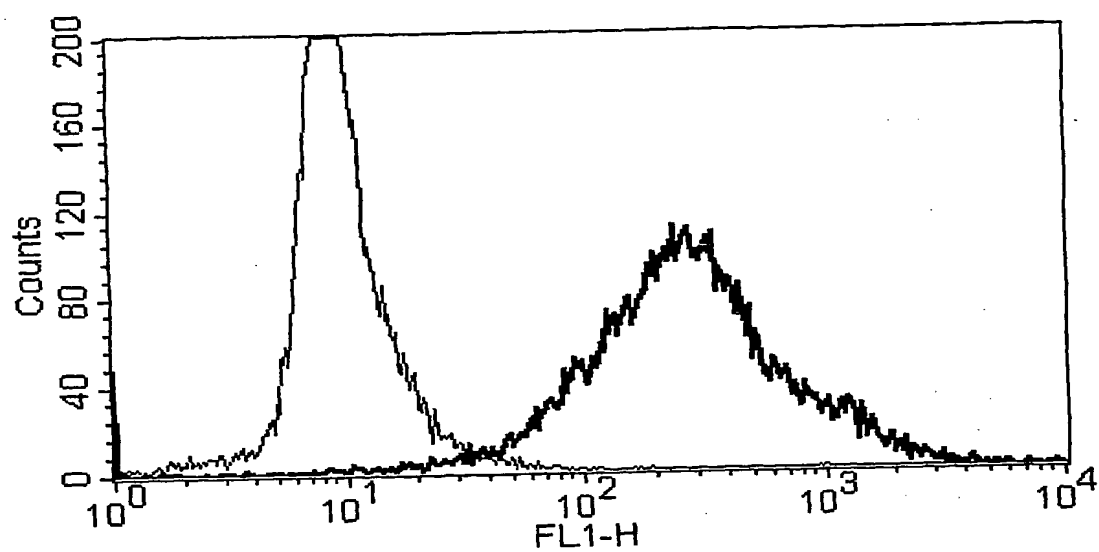
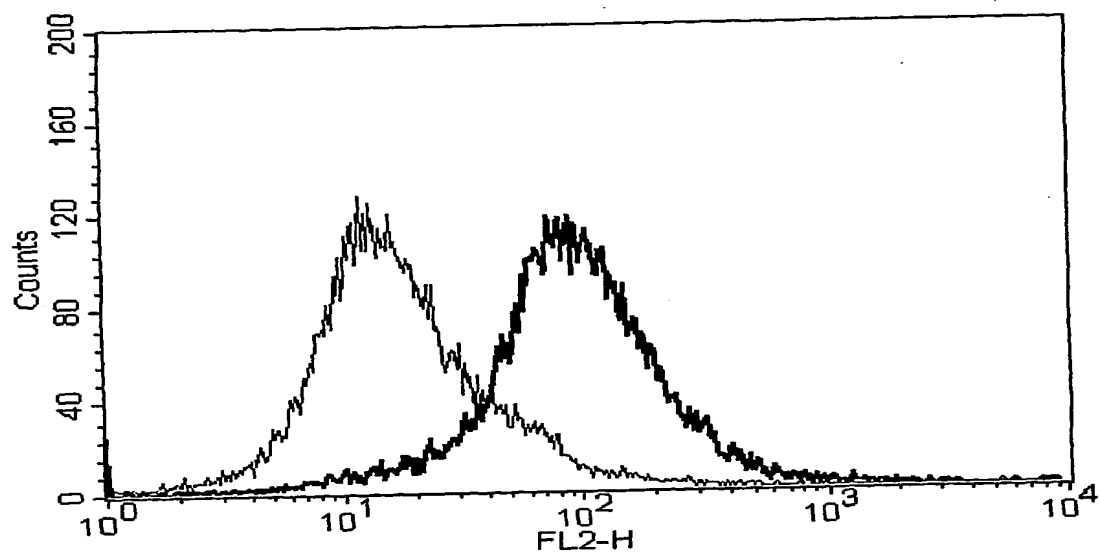
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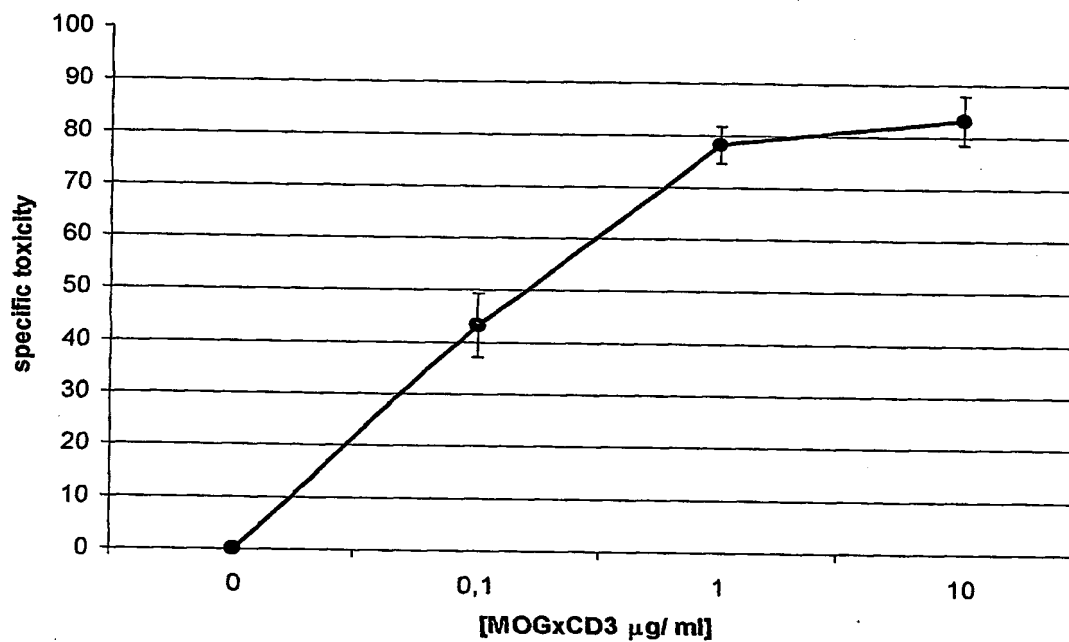
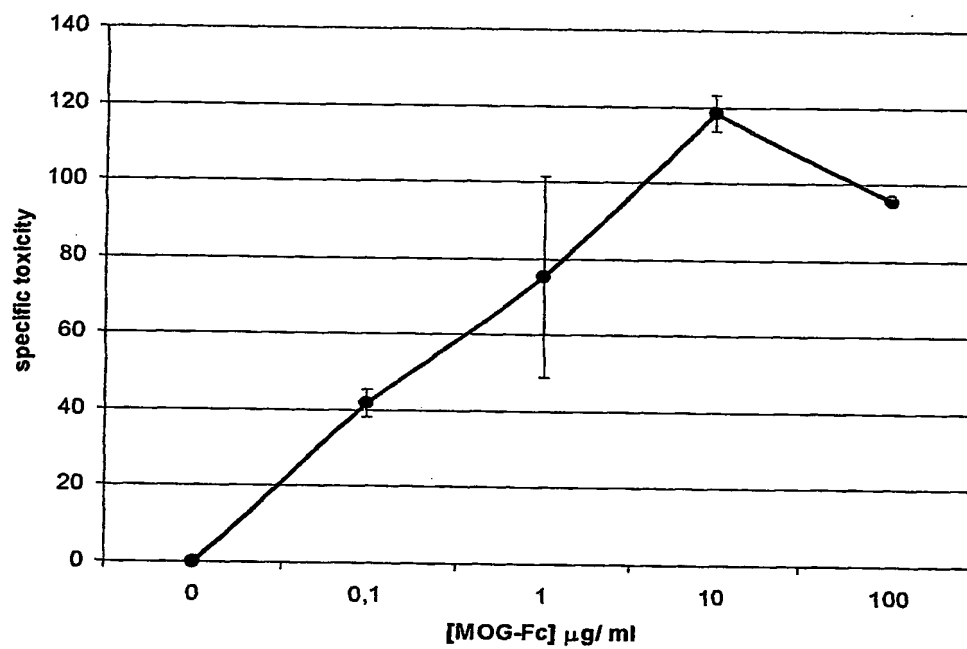
Fig. 9**A****B**

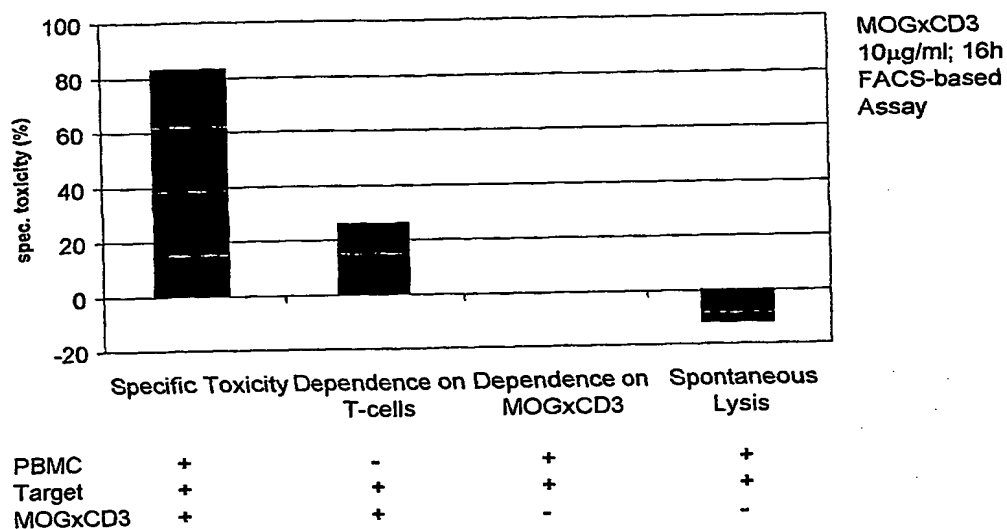
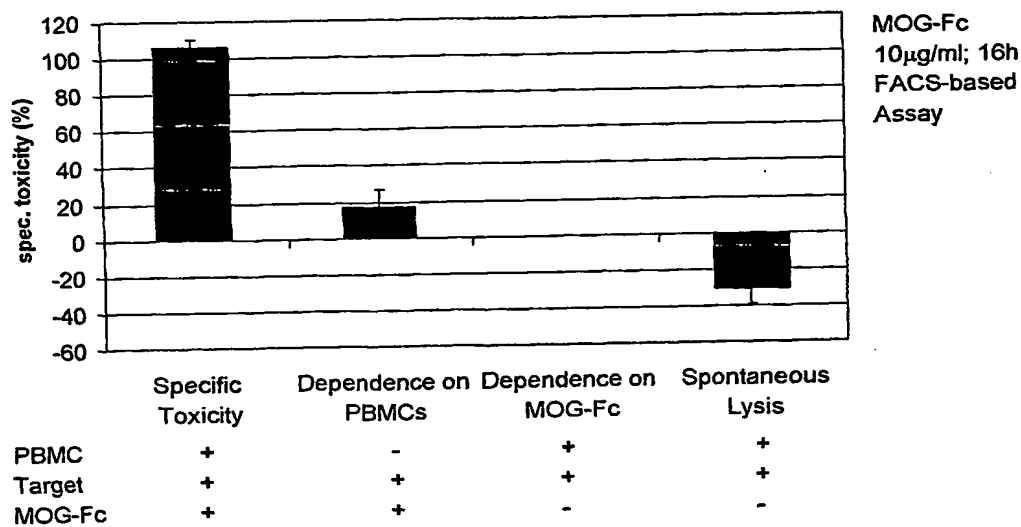
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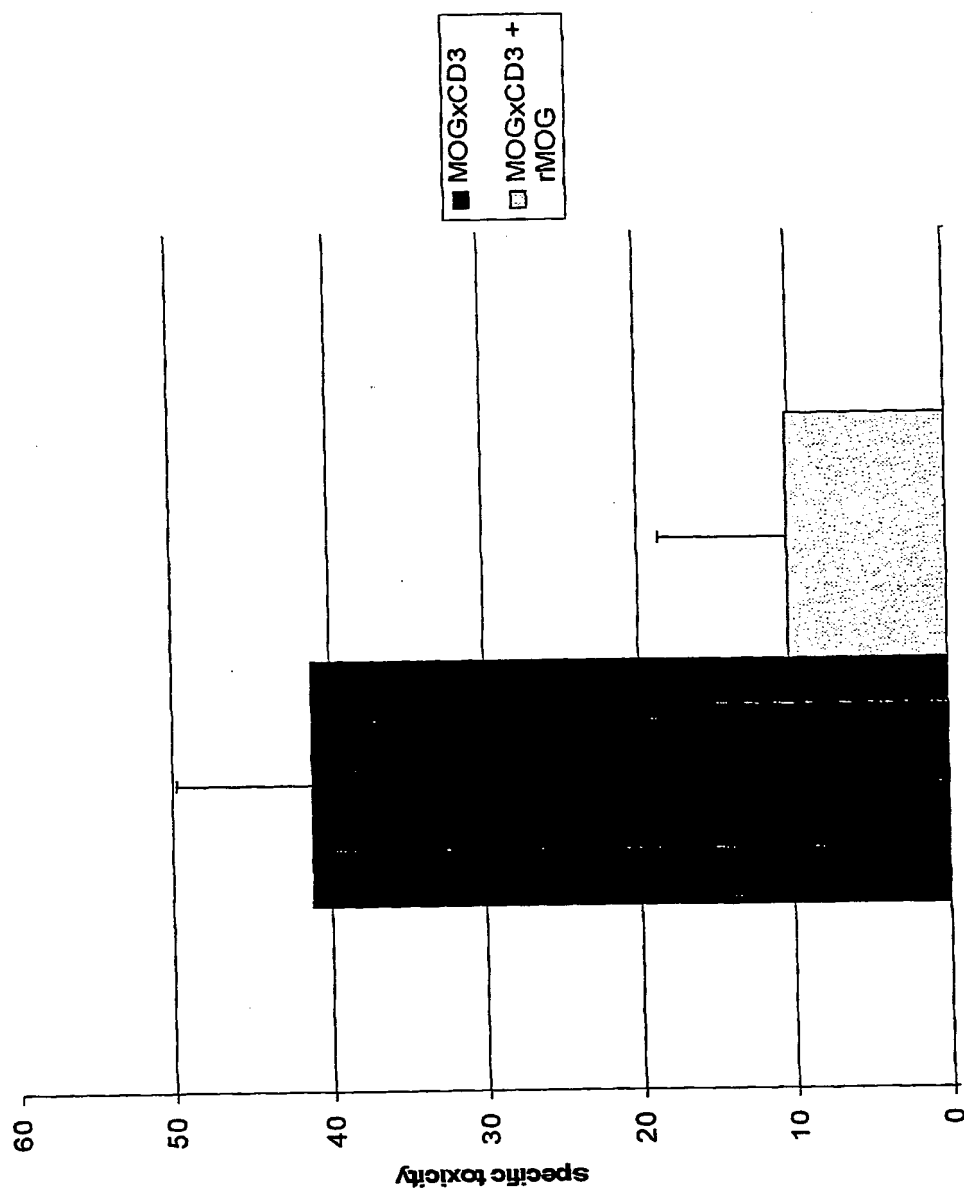
Fig. 11

Fig. 12

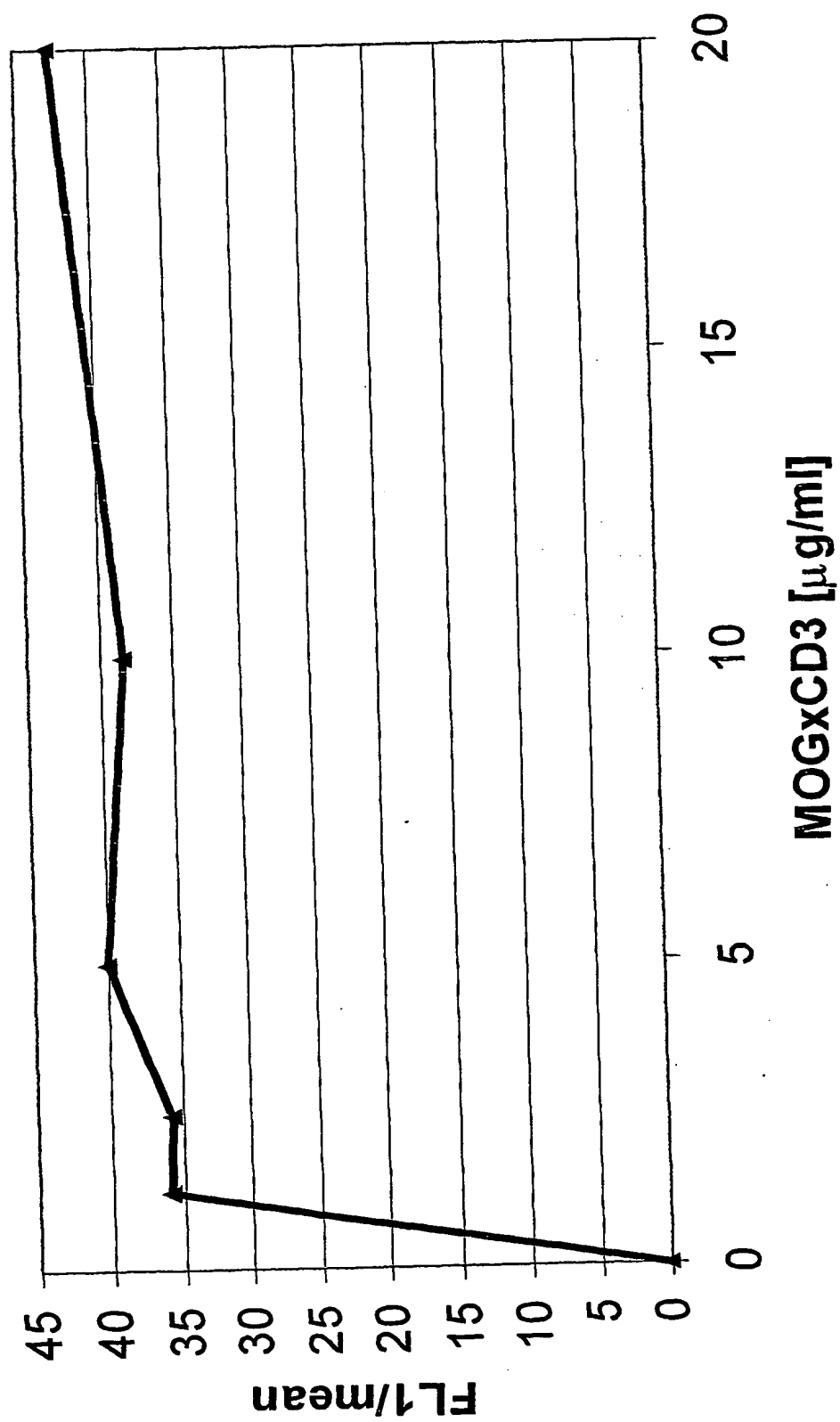


Fig. 13

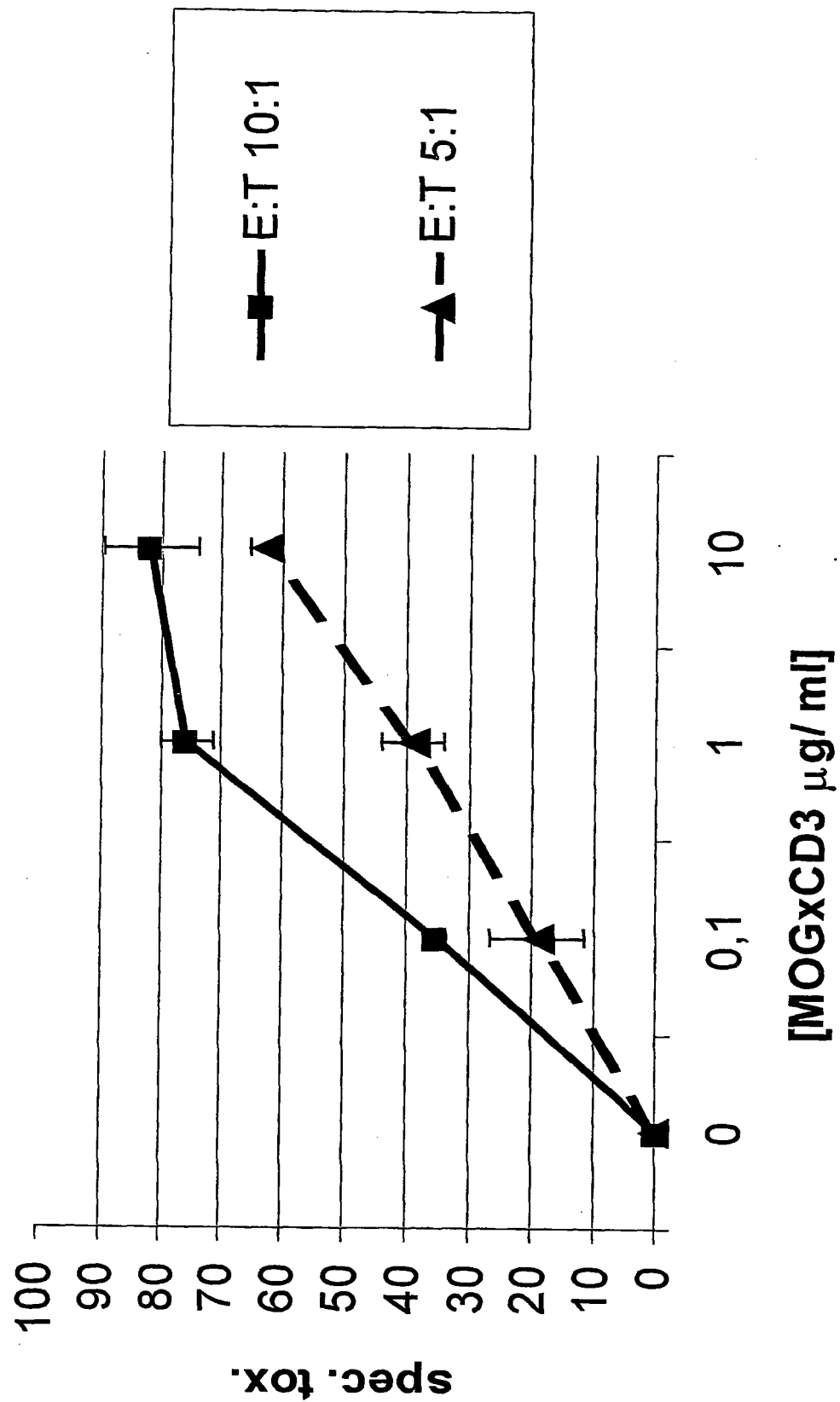
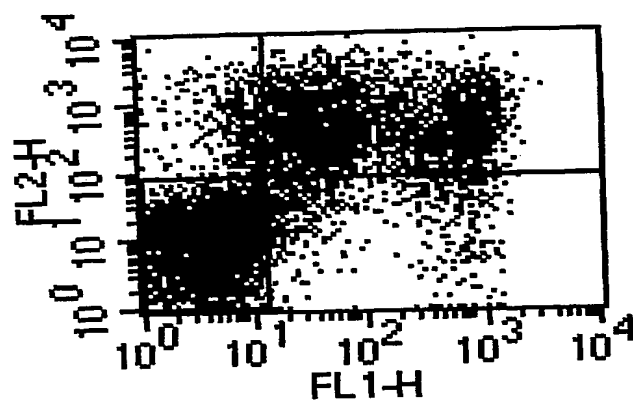
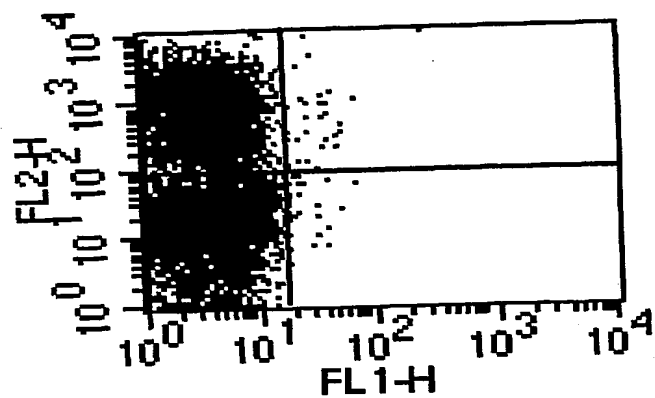


Fig. 14A

Specific binding of MOG-Fc fusion protein to IgM+ B-cells
derived from splenocytes from anti-MOG transgenic mice

A



IgM

huFc

Fig. 14B, C

Selective binding of MOG-Fc fusion protein to wildtype murine splenocytes

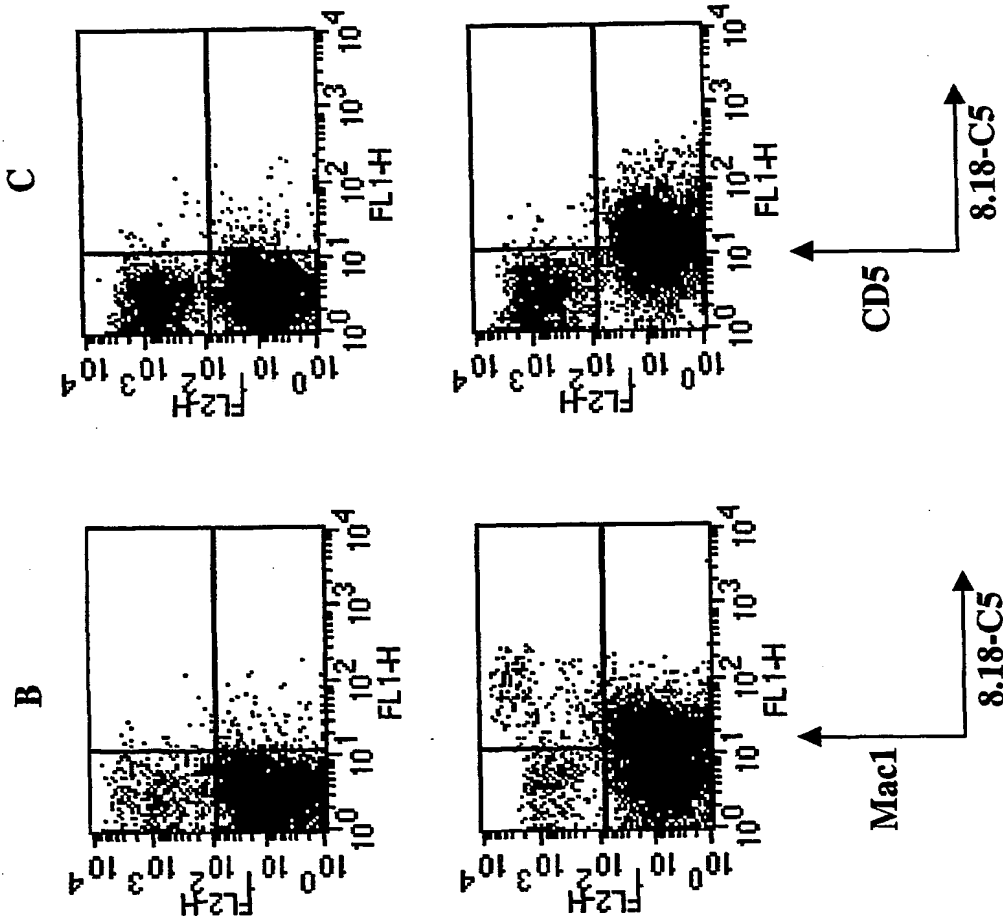


Fig. 14D

Specific binding of MOG-Fc fusion protein to murine macrophage cell line

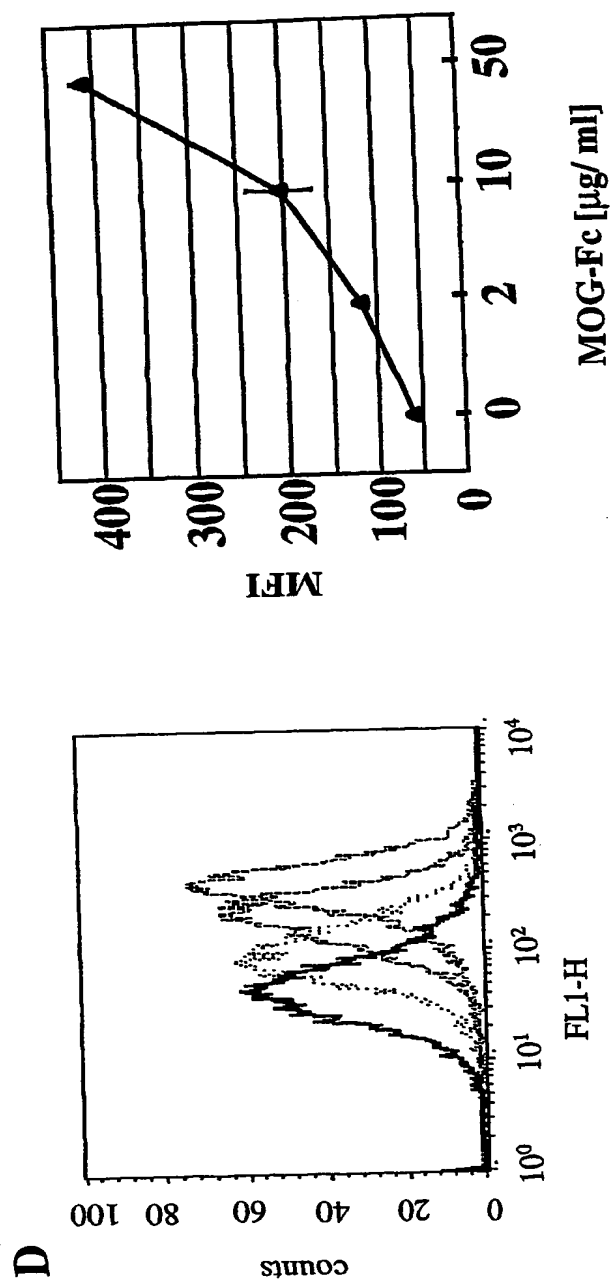


Fig. 15A

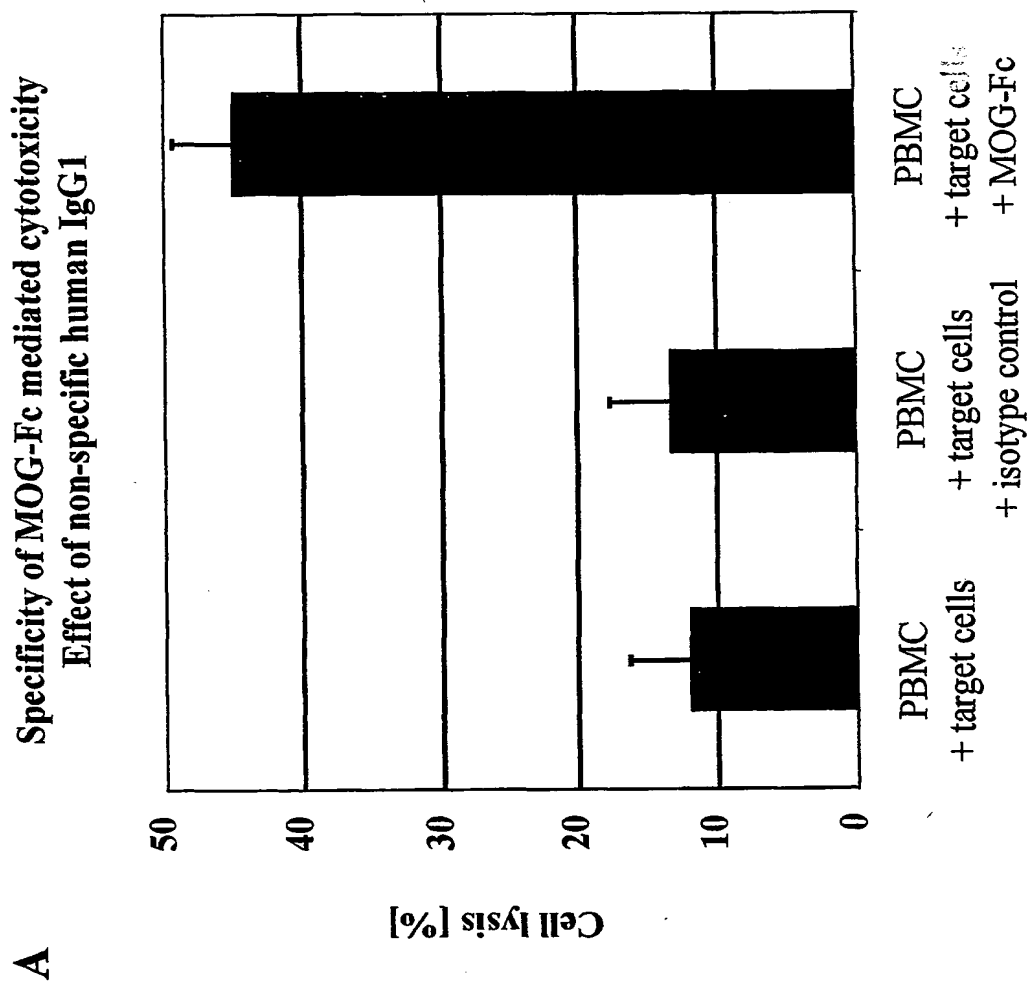


Fig. 15B

B
 Specificity of MOG-Fc mediated cytotoxicity
 Effect of unrelated murine cell line expressing cell surface IgG1

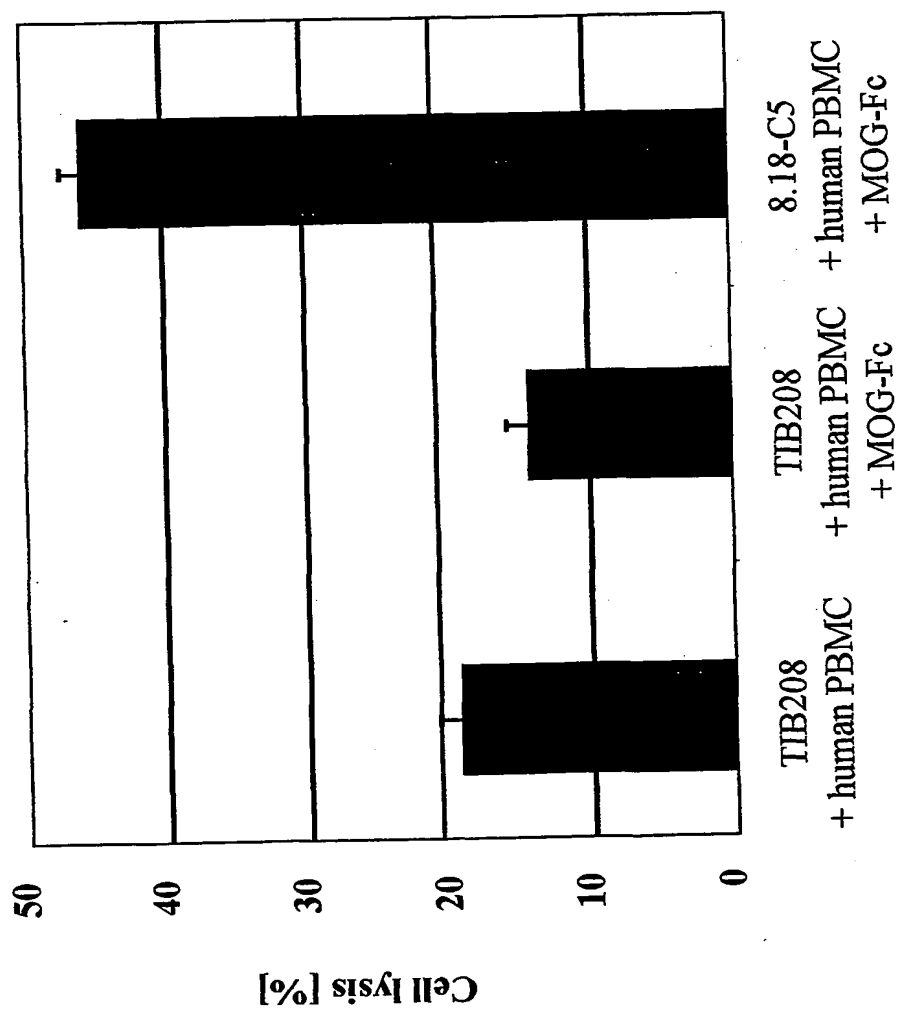


Fig. 16

Ex vivo depletion of B-cells by MOG-Fc

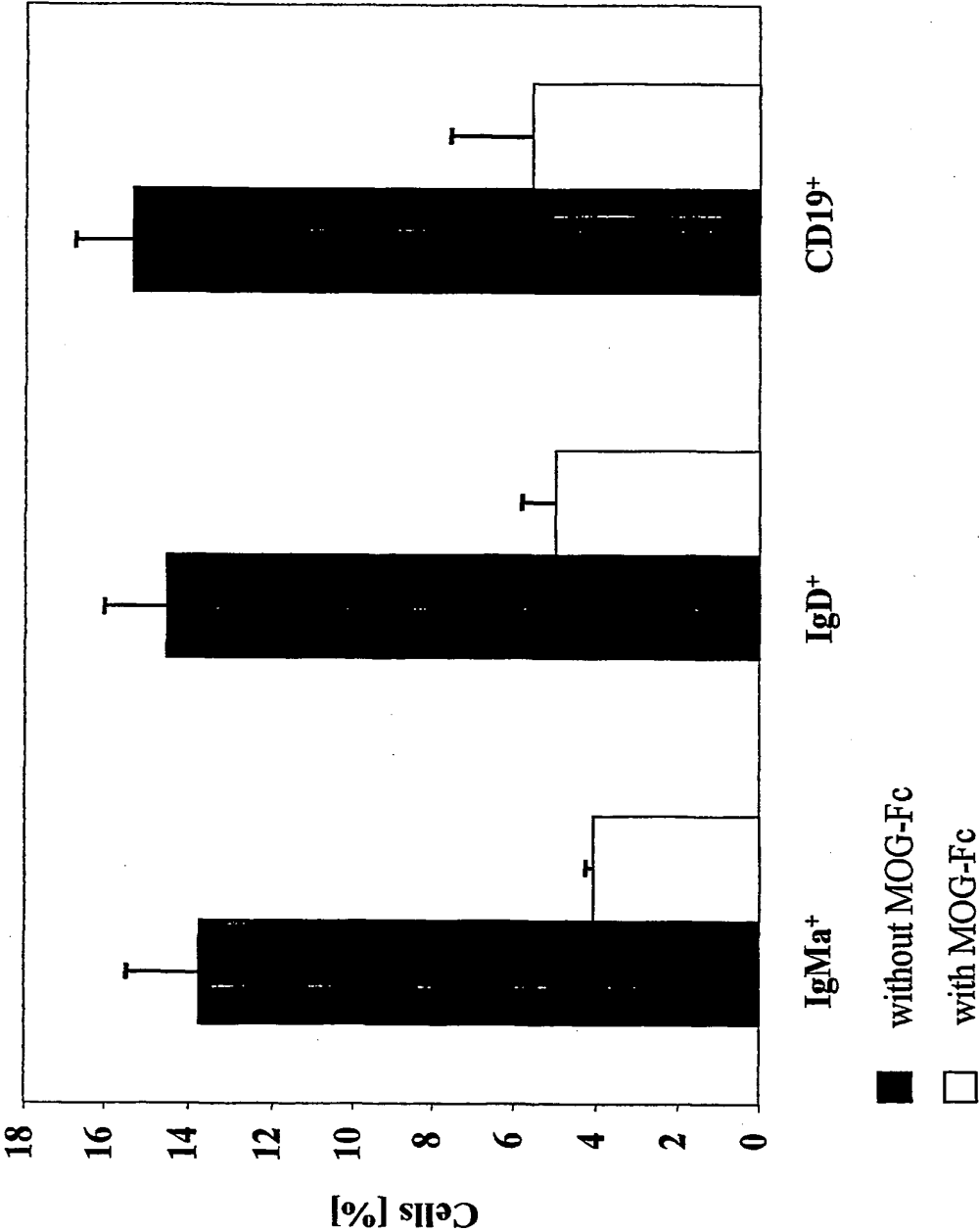


Fig. 1/A

In vivo depletion of anti-MOG specific B-cells in transgenic mice

A

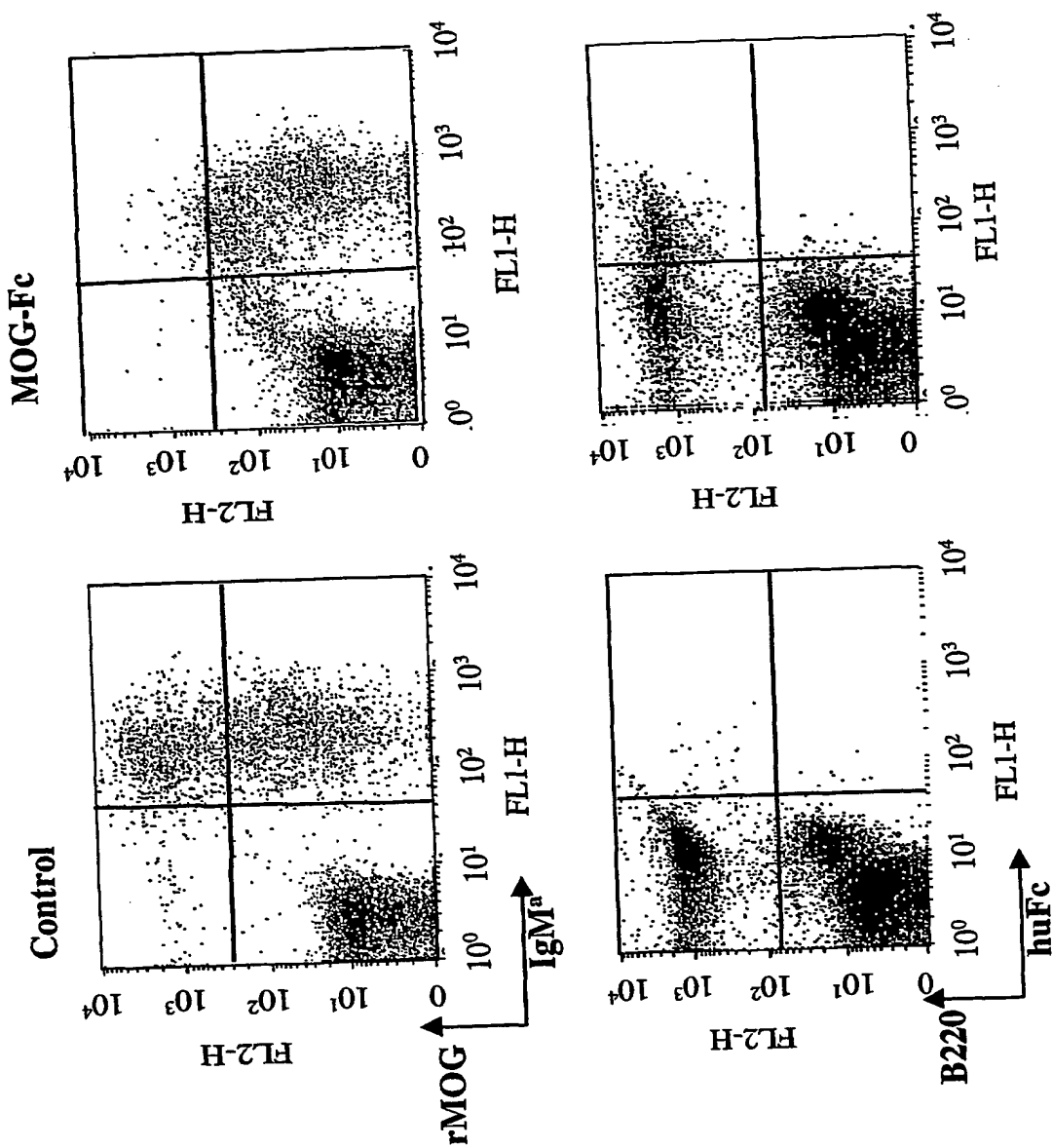


Fig. 17B

B In vivo depletion of anti-MOG specific B-cells in transgenic mice

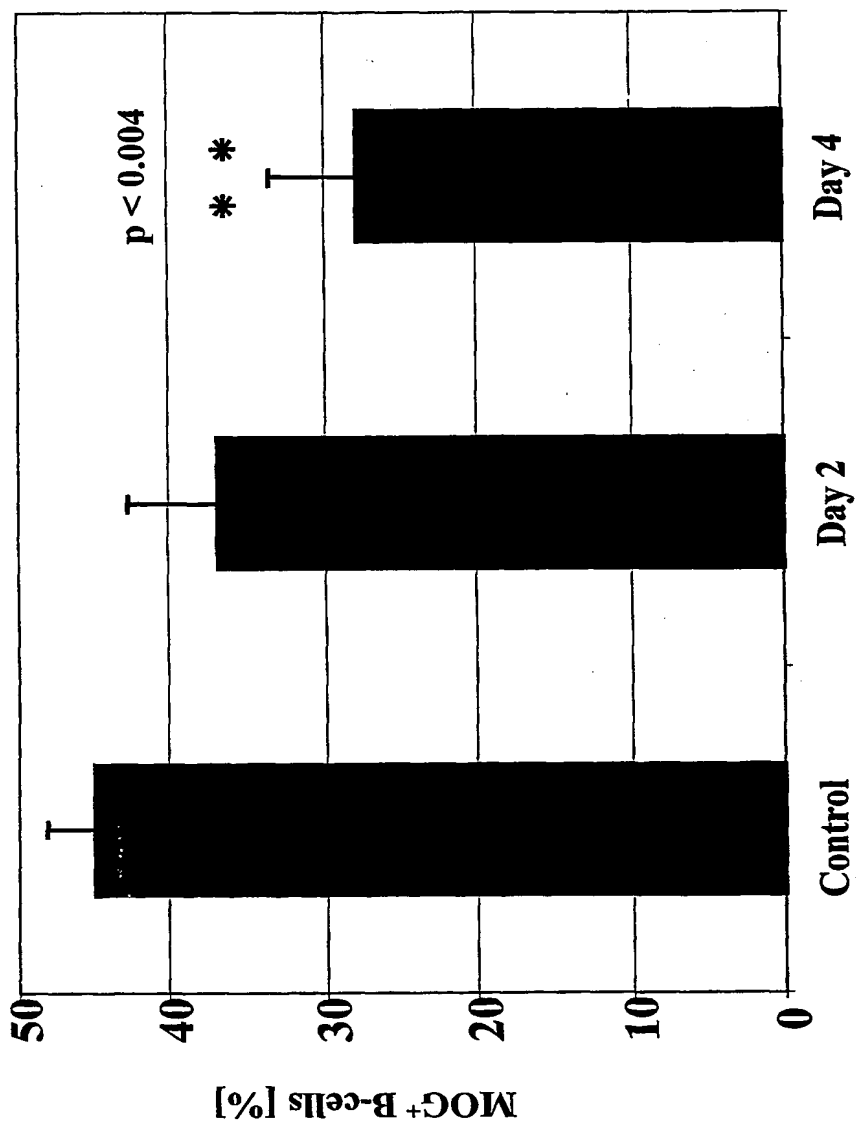


Fig. 18A

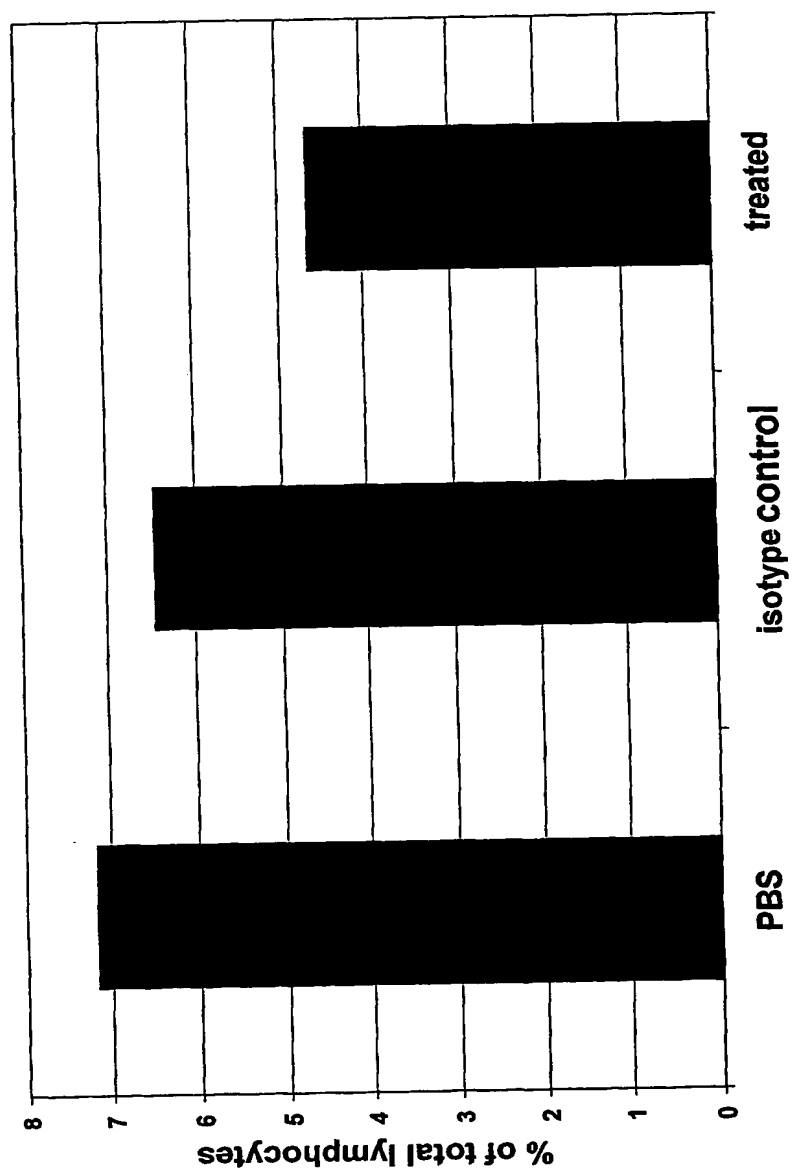


Fig. 18B

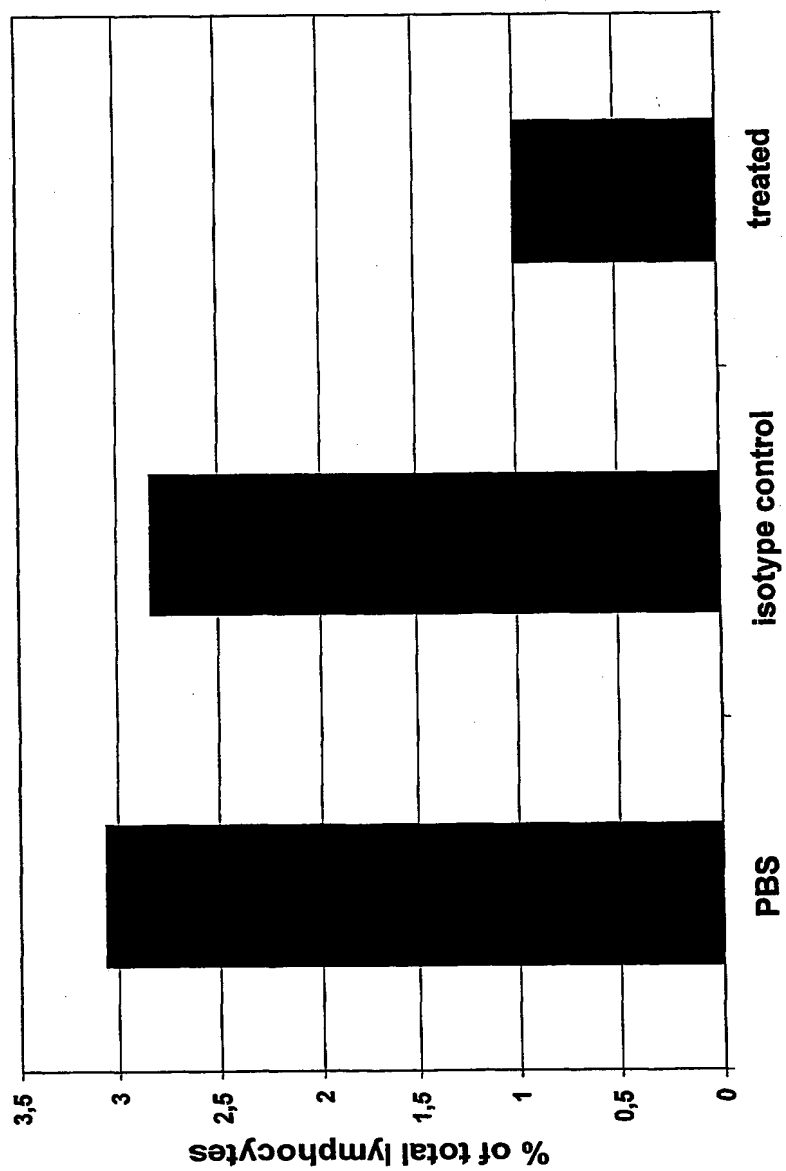


Fig. 19A

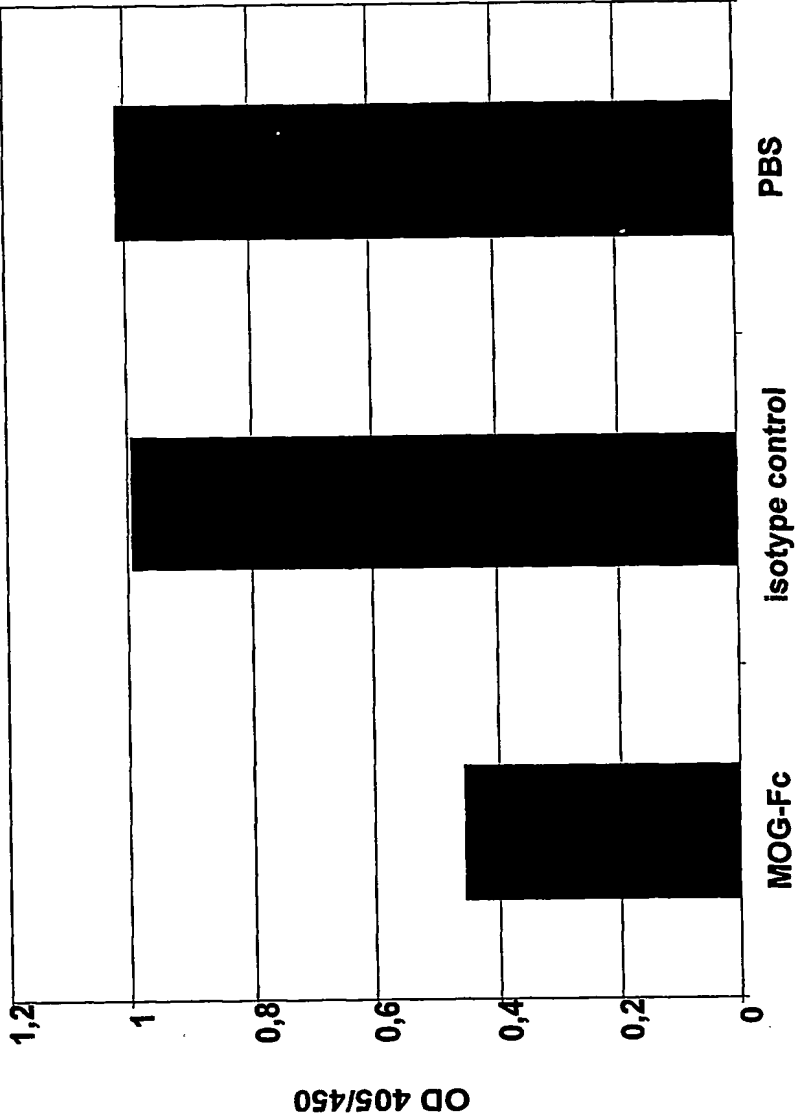
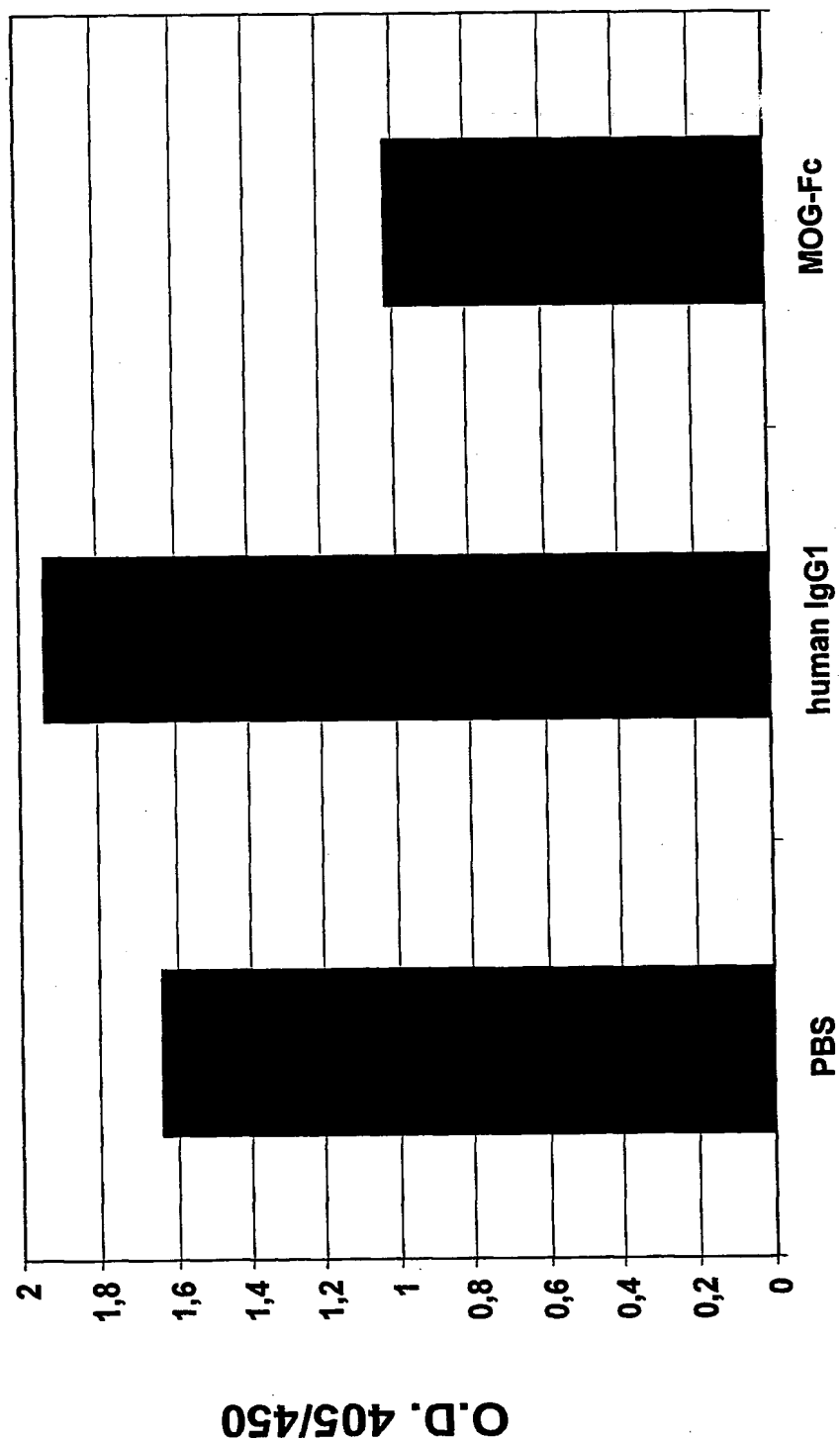


Fig. 19B



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 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 210 215 220
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 225 230 235 240
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 245 250 255
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 260 265 270
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 275 280 285
 Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 290 295 300
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 305 310 315 320
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 325 330 335
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 340 345 350
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

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28

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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

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31

<210> 8

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

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<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 9

tatccggaga gcccaaattct tgtgacaaaa c

31

6

5/6

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00117354.1 **22 August 2000 (22.08.2000) EP**

(71) Applicant (for all designated States except US): **MICROMET AG [DE/DE]**; Am Klopferspitz 19, 82152 Martinsried/München (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ZOCHER, Marcel [DE/DE]**; Theodor-Körner-Str. 13, 82049 München-Pullach (DE). **BÄUERLE, Patrick [DE/DE]**; Vogelsangstr. 13A, 82152 Gauting (DE). **DREIER, Torsten [DE/DE]**; Johann-Clanze-Str. 39, 81369 München (DE).

(74) Agent: **VOSSIUS & PARTNER**; Sieberstr. 4, 81675 München (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

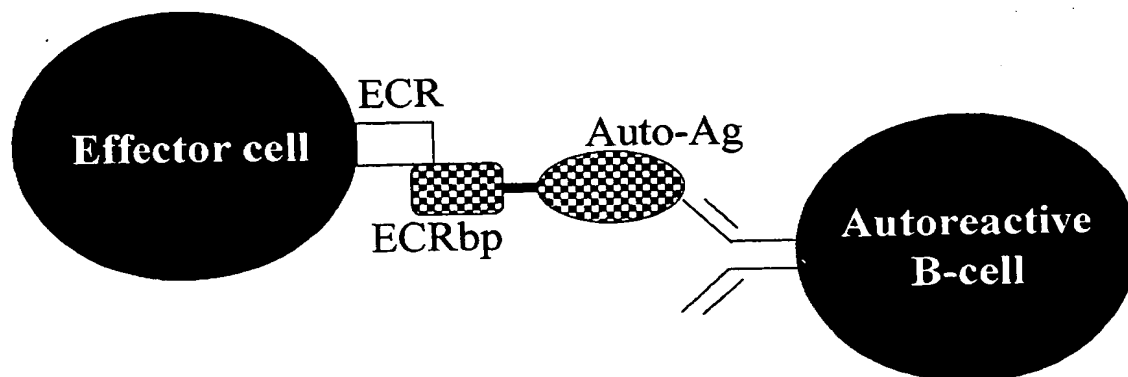
Published:

— with international search report

(88) Date of publication of the international search report:
7 November 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **COMPOSITION FOR THE ELIMINATION OF AUTOREACTIVE B-CELLS**



(57) Abstract: The present invention relates to a composition for the selective elimination of autoreactive B-cells comprising at least one (poly)peptide construct consisting of at least two domains wherein one of said domains comprises an autoreactive antigen or (a) fragments(s) thereof specifically recognized by the Ig receptors of said autoreactive B-cells and wherein one of said domains comprises an effector molecule capable of interacting with and/or of activating NK-cells, T-cells, macrophages, monocytes and/or granulocytes and/or capable of activating the complement system.

WO 02/016414 A3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/09714

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K19/00 C07K14/47 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, SEQUENCE SEARCH, CHEM ABS Data, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>DATABASE WPI Week 9722 Derwent Publications Ltd., London, GB; AN 1997-241758 XP002196726 "Phemphigus foliaceus antigen-IgG constant region fusion protein - linked through the hinge region used to treat pemphigus" & JP 09 077800 A (NISHIKAWA T), 25 March 1997 (1997-03-25) abstract</p>	<p>1-4, 16, 18, 23-26, 28, 29, 31-38</p>
Y		<p>5, 6, 13, 15, 17, 19-22, 27, 30</p>

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

15 July 2002

Date of mailing of the international search report

12.08.02

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Montrone, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/09714

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 99 23867 A (BIOGEN INC.; BROWNING JEFFREY (US)); 20 May 1999 (1999-05-20) cited in the application</p> <p>abstract page 1, line 11-24 page 3, line 6-9 page 4, line 24-26 page 7, line 9-17 page 11, line 3-7 page 22, line 6-14 page 24, line 21-23 page 27, line 14-26</p>	<p>1,2,4, 11,16, 18, 20-29, 31-38</p>
Y	<p>PROBY C M ET AL: "Development of chimeric molecules for recognition and targeting of antigen-specific B cells in pemphigus vulgaris." BRITISH JOURNAL OF DERMATOLOGY., vol. 142, no. 2, February 2000 (2000-02), pages 321-330, XP002196725 ISSN: 0007-0963 cited in the application</p> <p>abstract page 321, column 2, paragraph 2 page 322, column 1, paragraph 2; figure 1 page 326, column 2, paragraph 1 -page 328, column 2, paragraph 2</p>	<p>1-6,11, 13,15-38</p>
X	<p>WO 99 11667 A (RAYMACKERS JOSEPH ;UNION ANN (BE); INNOGENETICS NV (BE); MEHEUS LY) 11 March 1999 (1999-03-11) abstract page 2, line 3 -page 3, line 18 page 4, line 23-25 page 6, line 5-24 page 9, line 7-23 page 19, line 2 -page 20, line 14 page 33, line 8 -page 34, line 26</p>	<p>1-4,15, 16,23-38</p>
Y	<p>WO 95 24220 A (MEDAREX INC) 14 September 1995 (1995-09-14) abstract page 1, line 4 -page 2, line 37 page 4, line 16-23</p>	<p>1-6,11, 13,15-38</p>
Y	<p>US 5 932 448 A (TSO J YUN ET AL) 3 August 1999 (1999-08-03) abstract column 8, line 44 -column 10, line 48</p>	<p>1-6,11, 13,15-38</p>

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/09714

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHROEDER ET AL: "A recombinant bispecific single chain antibody CD19XCD3 induces rapid B cell lymphoma-directed cytotoxicity of unstimulated human T cells" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 92, no. 10, 15 November 1998 (1998-11-15), page 511A XP002115457 ISSN: 0006-4971 abstract	1-6,11, 13,15-38
A	WO 00 01732 A (UNIV TENNESSEE RES, CORP ;ZAGHOUBANI HABIB (US)) 13 January 2000 (2000-01-13) abstract page 10, line 15-19 page 15, line 27-33 page 28, line 4-26	1-4,11, 16,18, 20,33-38
P,Y	STEFFERL A ET AL: "Butyrophilin, a milk protein, modulates the encephalitogenic T cell response to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD.: 1950) UNITED STATES 1 SEP 2000, vol. 165, no. 5, 1 September 2000 (2000-09-01), pages 2859-2865, XP002206101 ISSN: 0022-1767 cited in the application abstract page 2859, column 1, paragraph 2 -column 2, paragraph 3 page 2862, column 2, paragraph 2 -page 2863, column 1, paragraph 3	1-4,13, 15-20, 23-38
Y	KHANNA A K ET AL: "Antibodies in the sera of acute rheumatic fever patients bind to human cardiac tropomyosin." JOURNAL OF AUTOIMMUNITY. ENGLAND FEB 1997, vol. 10, no. 1, February 1997 (1997-02), pages 99-106, XP002206102 ISSN: 0896-8411 cited in the application abstract page 99, column 1, paragraph 1 -column 2, paragraph 1 page 100, column 1, paragraph 1	1-4,13, 15-20, 23-38

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INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/EP 01/09714

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>LAKE D F ET AL: "Autoantibodies to the alpha/beta T-cell receptors in human immunodeficiency virus infection: dysregulation and mimicry."</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. UNITED STATES 8 NOV 1994, vol. 91, no. 23, 8 November 1994 (1994-11-08), pages 10849-10853, XP002206103 ISSN: 0027-8424 abstract page 10849, column 1, paragraph 1 -column 2, paragraph 1</p>	1-4,13, 15-20, 23-38
Y	<p>MATSUKURA H ET AL: "The Goodpasture antigen: common epitopes in the globular domains of collagen IV."</p> <p>NEPHRON. SWITZERLAND 1993, vol. 64, no. 4, 1993, pages 532-539, XP001088498 ISSN: 0028-2766 abstract page 532, column 1, paragraph 1 -page 533, column 1, paragraph 2</p>	1-4,6, 15-20, 23-38
Y	<p>BALDING S D ET AL: "Cicatricial pemphigoid autoantibodies react with multiple sites on the BP180 extracellular domain."</p> <p>THE JOURNAL OF INVESTIGATIVE DERMATOLOGY. UNITED STATES JAN 1996, vol. 106, no. 1, January 1996 (1996-01), pages 141-146, XP001087971 ISSN: 0022-202X abstract page 141, column 1, paragraph 1 -page 142, column 1, paragraph 3</p>	1-4,6, 15-20, 23-38
Y	<p>DÖPP R ET AL: "IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in Bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity."</p> <p>JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY. UNITED STATES APR 2000, vol. 42, no. 4, April 2000 (2000-04), pages 577-583, XP001087911 ISSN: 0190-9622 abstract page 577, column 1, paragraph 1 -column 2, paragraph 1 page 578, column 1, paragraph 3 -column 2, paragraph 1; figure 1 page 579, column 2, paragraph 3</p> <p style="text-align: center;">-/-</p>	1-4,6, 15-20, 23-38

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 01/09714

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DOMINGUEZ-LOPEZ MARIA LILI ET AL: "Cellular immune response to Klebsiella pneumoniae antigens in patients with HLA-B27+ ankylosing spondylitis." JOURNAL OF RHEUMATOLOGY, vol. 27, no. 6, June 2000 (2000-06), pages 1453-1460, XP001088217 ISSN: 0315-162X cited in the application abstract page 1453, column 1, paragraph 1 -column 2, paragraph 2 page 1459, column 1, paragraph 3	1-4, 13, 15-20, 23-38
Y	OBERMAYER-STRAUB P ET AL: "Target proteins in human autoimmunity: cytochromes P450 and UDP-glucuronosyltransferases." CANADIAN JOURNAL OF GASTROENTEROLOGY = JOURNAL CANADIEN DE GASTROENTEROLOGIE. CANADA MAY 2000, vol. 14, no. 5, May 2000 (2000-05), pages 429-439, XP001088216 ISSN: 0835-7900 cited in the application abstract page 430, column 1, paragraph 1 -page 431, column 2, paragraph 3 page 437, column 1, paragraph 2 -column 2, paragraph 1	1-4, 11, 15-20, 23-38
Y	EYSTATHIOY THEOPHANY ET AL: "Human autoantibodies to a novel Golgi protein golgin-67: High similarity with golgin-95/gm 130 autoantigen." JOURNAL OF AUTOIMMUNITY, vol. 14, no. 2, March 2000 (2000-03), pages 179-187, XP001088204 ISSN: 0896-8411 cited in the application abstract page 179, column 1, paragraph 1 -page 180, column 1, paragraph 1 page 183, column 1, paragraph 2 page 185, column 1, paragraph 2 --- -/-	1-4, 11, 15-20, 23-38

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/EP 01/09714

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOCK R J ET AL: "IgA anti-tissue transglutaminase as a diagnostic marker of gluten sensitive enteropathy." JOURNAL OF CLINICAL PATHOLOGY, (LONDON); vol. 52, no. 4, April 1999 (1999-04), pages 274-277, XP001087690 ISSN: 0021-9746 cited in the application abstract page 274, column 2, paragraph 1 -----	1-4, 13, 15-20, 23-38
Y	EBRINGER A ET AL: "HLA molecules, bacteria and autoimmunity." JOURNAL OF MEDICAL MICROBIOLOGY. ENGLAND APR 2000, vol. 49, no. 4, April 2000 (2000-04), pages 305-311, XP001087834 ISSN: 0022-2615 cited in the application abstract page 305, column 1, paragraph 2 page 306, column 2, paragraph 3 -page 307, column 1, paragraph 2 page 308, column 2, paragraph 1 -----	1-4, 13, 15-20, 23-38
Y	LINK B K ET AL: "Production and Characterization of a Bispecific IgG Capable of Inducing T-Cell-Mediated Lysis of Malignant B Cells" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 81, no. 12, 15 June 1993 (1993-06-15), pages 3343-3349, XP002900864 ISSN: 0006-4971 abstract page 3343, column 2, paragraph 2 page 3348, column 1, paragraph 3 -----	1-6, 11, 13, 15-38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/09714

Box I Observations where certain claims were found unsearchable. (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 37 and 38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-4(all partially), 5, 6, 11, 13(all completely), 15-20(all partially), 21, 22(all complete), 23-38(all partially)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4 (all partially), 5, 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of an intracellular autoreactive matrix protein antigen and a domain which allows interaction with an effector cell.

2. Claims: 1-4 (all partially), 6, 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of an extracellular autoreactive matrix protein antigen and a domain which allows interaction with an effector cell.

3. Claims: 1-4 (all partially), 7, 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of a complement autoreactive protein antigen and a domain which allows interaction with an effector cell.

4. Claims: 1-4 (all partially), 8, 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of nuclear autoreactive antigens and a domain which allows interaction with an effector cell.

5. Claims: 1-4 (all partially), 9, 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of cell surface receptor autoreactive antigens and a domain which allows interaction with an effector cell.

6. Claims: 1-4 (all partially), 15-20 (all partially),
23-38 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210.

A composition for the selective elimination of autoreactive B cells consisting of nuclear receptor autoreactive antigens and a domain which allows interaction with an effector cell.

7. Claims: 1-4 (all partially), 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of lipid autoreactive antigens and a domain which allows interaction with an effector cell.

8. Claims: 1-4 (all partially), 10, 15-20 (all partially),
23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of soluble factor autoreactive antigens and a domain which allows interaction with an effector cell.

9. Claims: 1-4 (all partially), 11 (complete),
15-20 (all partially), 21 (complete),
22 (complete), 23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of membrane protein autoreactive antigens and a domain which allows interaction with an effector cell.

10. Claims: 1-4 (all partially), 12 (complete),
15-20 (all partially), 23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of heat shock protein autoreactive antigens and a domain which allows interaction with an effector cell.

11. Claims: 1-4 (all partially), 13 (partially),
15-20 (all partially), 23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of autoreactive antigens derived from proteins with sequence similarity to microbial antigens and a domain which allows interaction with an effector cell.

12. Claims: 1-4 (all partially), 13 (partially),

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210.

15-20 (all partially), 23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of autoreactive antigens derived from proteins with sequence similarity to dietary components and a domain which allows interaction with an effector cell.

13. Claims: 1-4 (all partially), 14 (complete),
15-20 (all partially), 23-38 (all partially)

A composition for the selective elimination of autoreactive B cells consisting of autoreactive antigens derived from proteins with sequence similarity to proteins of intercellular structures and a domain which allows interaction with an effector cell.

INTERNATIONAL SEARCH REPORT
on patent family members

International Application No
PCT/EP 01/09714

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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WO 9923867	A	20-05-1999	AU 1389999 A CA 2308440 A1 EP 1029049 A2 JP 2001522589 T WO 9923867 A2 ZA 9810133 A	31-05-1999 20-05-1999 23-08-2000 20-11-2001 20-05-1999 17-05-1999
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